STUDIES ON LEMONGRASS OIL

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CERTIFICATE

Certified that this thesis is based on the work done by Mrs. K.N.Pushpakumari under my guidance in the Department of Applied Chemistry, Cochin University of Science and Technology and no part of this has been presented by her for any other degree.

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DECLARATION

Certified that the work presented in this thesis is based on the original work done by me under the guidance of Dr. Paul A.Vatakencherry, Professor & Head, Department of Applied Chemistry, Cochin University of Science and Technology and has not been included in any other thesis submitted for the award of any degree.

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C O N T E N T S

C H A P T E R I

INTRODUCTION TO LEMONGRASS OIL

1. Introduction

The oil of lemongrass (Cymbopogon flexuosus) is one of the most important essential oils. It will continue to be one of the "big ten" of our essential oils[1]. Lemongrass oil is obtained from certain species of grasses of the genus cymbopogon. The genus consists of about 80 species, 10 to 12 of which are known to occur in India. Lemongrass is a stoloniferous plant. The plant grows wild in many tropical and semitropical parts of Asia, Africa and in parts of Central America and South America. For the extraction of the oil however only cultivated lemongrass is employed. The trade distinguishes two principal types of lemongrass oil, viz. the East Indian Oil and West Indian Oil. There was much confusion, years ago, about the taxonomy of the plants which yield the East Indian and West Indian types of lemongrass oil, however Stapf[2] ended the long controversy of identifying the plant yielding the East Indian type oil as Cymbopogon flexuosus (D.C.) Stapf and the plant yielding the West Indian type oil as Cymbopogon citratus (D.C.) stapf. The 2 plants have been named variously also Andropogon nardus var. Flexuosus Hack or A. citratus D.C. respectively.

The correctness of Stapf's classification was confirmed experimentally by Jonitt and Pickles[3]. Experiments carried out at the Government station in Barbados[4] (B.W.I.) with East Indian lemongrass seed imported from the State of Cochin, now part of the state of Kerala, on the Malabar coast indicated that grass raised from this seed yielded an oil which was readily soluble in alcohol and contained a high percentage of citral. Hood[5] does not contest the claim of Stapf that there are two distinct species of lemongrass, viz. C. flexuosus and C. citratus but assumes the existence of numerous local varieties of C. citratus. Both these types contain 70-80% of aldehyde (citral), but the two oils differ slightly in their solubility in alcohol. The West Indian Oil is usually less soluble in 70% alcohol than the East Indian Oil. The lower solubility of the West Indian Oil, particularly after storage of freshly distilled oil, is due to the presence of myrcene an olefinic terpene which on exposure to air and light polymerises readily. The solubility in alcohol is regarded as synonymus with freshness and freedom from adulterants; though investigations have disclosed that some specimens of freshly distilled oil are also found to be insoluble in alcohols. Because of the uniformly high citral

content and solubility in 70% alcohols, the East Indian oil is preferred in the World market of perfumery trade. Table - I summarises the physical properties of East Indian and West Indian type oils.

1.1. Oil of West Indian Lemongrass (C. Citratus Stapf - Syn. Andropogon Citratus D.C. - Vasanapullu)

The citratus grass native to Srilanka, is now produced in large quantities in several parts of the world like Guatemala, Haiti (West Indies), Sao Paulo (Brazil), the Comoro Islands, Madagascar and Indo-China. More or less successful experiments in cultivating the plant and distilling the oil have been undertaken in Puerto Rico and Dominica, Mexico and even in places like Florida (U.S.A.). In India, West Indian lemongrass is grown in the gardens of Punjab, Bombay and Baroda. A small quantity is produced in Java and Ceylon. The grass is not used for the production of oil in India. It is chiefly used in India for flavouring soups and curries. Cymbopogon citratus is propagated through division of clumps. An infusion of grass is sometimes taken as a refreshing beverage and this use gives it the name "Hirvacha" or "Green tea". In Java, it is used in the preparation

Table - I

Source	Yield %	Specific gravity	Refractive index	Principal constituent	Solubility
East Indian C.Flexuosus	0.3 to 0.5	0.804 - 0.908	1.483 - 1.489	65 - 85%	Soluble in 3 Vols. of 70% alcohol.
West Indian C.Citratus	0.2	0.869 - 0.894	1.483 - 1.489 ($20^{o}C$)	57 - 84%	Only slightly soluble

of a highly spiced sherbet. The principal constituent is citral, together with other constituents, like citronellal, geranial and myrcene.

1.2 Oil of East Indian Lemongrass [C.Flexuosus Syn. Andropogon Nardev var. Flexuosus Hack (Kodipullu)]

C. Flexuosus is indegenous to India. The oil from it is also known as Cochin or Malabar lemongrass oil (Malayalam-Pulthailam) and it is propagated through seeds. The grass is found in Tinnevelli and in the Travancore and Cochin part of Kerala. It is known in the trade for over 150 years. The same East Indian Oil of lemongrass has also been retained as a mark of differentiation in view of the fact that a large part of Indian oil is exported in essential oil trade abroad and hence the name is well established. Only the East Indian Lemongrass (Cymbopogon flexuosus) oil will be considered in the discussion which follows.

1.3 Producing regions

India produces the largest quantity of lemongrass oil in the world, 80% of it being in Kerala. East Indian Oil is mainly produced in Kerala State and a small portion of the oil is produced in Karnataka State. Most of the plantations are located in the foot

hills of ghats, a range of high and wild mountains stretching inland from north to south and paralleling Malabar coast.

The northern sections of the producing regions, from which bulk of the oil originates, produce a better quality of the oil than the southern districts. During 1950-60, the major producing places were the villages of Thodupuzha, Vazhakulam, Muvattupuzha, Perumbavoor, Alwaye and Kothamangalam. Now the situation has slightly changed and the northern districts Cannanore, Palghat and Calicut are the major producing regions, the main region being around Attapadi. Cochin and Kothamangalam are the important trading centres.

1.4 Soil, Climate and Altitude[8,9]

Lemongrass is a crop admirably suited to the waste lands and hilly slopes of the West coast, where no annual crop would grow. It requires a warm tropical climate, plenty ofsunshine and intermittent but not excessive rainfall. The grass grows best on well drained sandy loam or loose laterite soil. The type of the soil and the humidity condition of the atmosphere have a considerable influence on the quality and quantity of the oil produced. Well drained arenaceous soil produces lemongrass plants with higher yield of oil having higher citral content. With

increase in humidity (during heavy rains) the yield of the oil decreases as also of citral to some extent. Fertile soil are known to produce oil with lower citral content. Plants from sandy soil yield relatively more oil of higher citral content than plants from very fertile soil. Most of the lemongrass plantations are located on low priced waste lands on the slopes of hills, upto 3000 ft. altitude. The average holdings are scattered widely and range in area from small patches to 4-5 hectares. On an average one acre yields about 100 kg of oil per year with 4 cuttings. Higher yields may be obtained on the fields that are well managed.

1.5 Area under cultivation[9]

Although in the past cultivation of lemongrass was solely confined to Kerala of late cultivation of lemongrass has extended to areas in Karnataka and Tamilnadu bordering on Kerala. The total area under cultivation of lemongrass is estimated to be about 4,000 hectares. Out of this area, Kerala accounts for about 3,500 hectares, Karnataka about 350 hectares and Tamilnadu about 150 hectares. It is reported that the soil conservation department, Government of Orissa has brought about 50 hectares in Koraput district

under the cultivation of lemongrass the improved variety O.D. - 19.

The area under lemongrass cultivation steadily increased from 1954 onwards due to increased demand of the oil from abroad. The maximum area under lemongrass cultivations around the year 1963-64 was 8,000 hectares. Thereafter the demand for lemongrass oil began to shrink and the area under cultivation also correspondingly came down.

Preference for lemongrass cultivation depends on two important factors viz. remunerative price and availability of land for this purpose. If a crop other than lemongrass can be cultivated more profitably; lemongrass is ignored. If the land is not suitable for cultivating more profitable crops, lemongrass is cultivated on such land. Where lemongrass is cultivated nothing else grows on that land or near about that land. This is another hazard in the cultivation of lemongrass.

Over the decades, cultivation of lemongrass in Kerala has been shifting from the coastal areas into the interior, particularly in the northern districts. This is due to the irrigational facilities provided in the coastal areas are utilised for more profitable crops. The invasion of rubber plantations

in these areas has also contributed to this shift in a significant measure. For example, the importance of Angamali, Perumumbavur, Kuruppampadi and Muvattupuzha has reduced and other areas have come under lemongrass cultivation in Kerala. In recent years, Idikki and Rajakad in the interior Wynad have become more important areas for lemongrass cultivation. The important places for lemongrass cultivation are now Tellicherry, Mananthawadi, Nilambur, Ernad, Peravoor, Tamarassery and Wynad in North Kerala; Coorgy and south Kanara in Karnataka and Uthakamandalam and Kanyakumari districts in Tamilnadu.

More than 80% of the land on which lemongrass is cultivated is unused land belonging to Government Hardly 15 to 20% of the lemongrass is cultivated on land belonging to the growers.

1.6 Plant varieties[8,9]

There are two types of lemongrass in Kerala differing in appearance and readily distinguishable by the colour of the stems.

1. In the case of the so called "Red Grass" locally known as "Chomannapullu", the colour of the stem is red. The leaves of this plant, the true Cymbopogon flexuosus Stapf, yield the normal East Indian

lemongrass oil containing 75% or more of aldehyde, mainly citral and exhibiting good solubility in 70% alcohol. The bulk of the Indian lemongrass oil is produced from the red grass.

2. The white stemmed variety, the so called "White Grass" is locally known as "Vellapullu". The oil derived from this plant contains low percentage of aldehydes and has poor solubility in 70% alcohol. This plant has been identified as Cymbopogon flexuosus Stapf albescenes[10]. The oil from the white variety contains camphene, dipentene and no citral[11]. Therefore the oil from the white grass cannot be marketed as normal lemongrass oil but is used as an adulterant of lemongrass oil for adjusting the aldehyde content. This variety has been almost rooted out of lemongrass fields by intensive efforts of the state departments of Agriculture and forests.

The following constants are obtained from two typical samples of oils from red grass and white grass respectively. (See Table II).

Table - II

Specifications	Oil from Red grass (C.Flexuosus Stapf)	Oil from White grass(C.Flexuosus Stapf albescenes)
Specific gravity	0.881	0.931
Refractive index at 30°C	1.482	1.498
Aldehyde content	76.4%	8.9%
Solubility in 70% Alcohol % by weight	2.8	Insoluble

1.7 Improved variety of Red Grass

The two main varieties of Red grass which are cultivated in Kerala are the local one and O.D. - 19[1]. O.D. - 19 being the improved variety, developed at the lemongrass research station at Odakkali, near Perumbavur, Kerala. Their comparative performance is given in Table - III.

Transplanted crop is found to be superior to directly sown crop in respect of yield of lemongrass oil and citral content in the oil. Depending upon the soil and climatic conditions the crop can be retained in the field for 5 to 6 years. The yield of oil per hectare during second to sixth year is 80 to 100 kg per year. The time required for steam distillation of one charge is 2 to 2½ hours for charging the still and discharging the plant material[5].

1.8 Physico Chemical Properties

The most important properties of lemongrass oil that are usually considered are specific gravity, refractive index, optical rotation, solubility in alcohol and percentage of citral. Oil of East Indian lemongrass is a dark yellow to reddish brown liquid. It has a characteristic sharp, pungent lemon like odour because of large amount of citral present.

Table - III

	O.D. - 19	Local
Average yield per annum	20 Mt/Ha	12 Mt/Ha
Average yield of oil	80 kg/Ha	40 kg/Ha
Percentage of oil recovery	0.4%	0.32%
Citral content of the oil calculated as total aldehyde content	85 - 90%	75 - 80%

A decrease in citral content, solubility and increase in density have been observed in lemongrass oil during storage. These changes occur rapidly if the oil contains moisture. Lemongrass oil should be thoroughly dried and stored in air tight containers.

The characteristic properties for East Indian lemongrass oil is summarised in Table - IV.

Besides citral, other reported constituents of East Indian lemongrass oil are given below[13].

Dipentene	limonene
myrcene	geraniol
nerol	methyl heptenol
linalool	citronellol
farnesol	n-decylaldehyde
citronellal	methyl heptenone
geranyl acetate[13]	

Table - V summarises the constituents of lemongrass oil with their physical constants. The gas liquid chromatographic analysis is also done and some of the peaks are identified. Undoubtedly East Indian lemongrass oil contains quite a number of other components in traces amount, which are not identified.

Table IV[12]

Sl. No.	Properties	Expected Range	Method of test Ref. to CL-No. in IS-326-1952
1.	Colour and appearance	Dark yellow to light brown red mobile liquid	41
2.	Odour	Lemon like	4.1
3.	Specific gravity	0.888 - 0.898	5
4.	Optical Rotation	$+3^{o}$ to $+1^{o}$	6
5.	Refractive index at 30°C	1.4786 to 1.4846	7
6.	Percentage of citral content by vol. min. (estimated by using freshly prepared 30% solution of sodium bisulphite)	75	..

Table - V

Constituents of Lemongrass Oil (in the increasing order of Boiling Points)

	Name	Formula	Molecular weight	B.P/760 mm	Refracfive index	Density	Uses
1.	Myrcene	$C_{10}H_{16}$	136.23	171.5°	1.4709	d^{20} - .794	Intermediate in the manufacture of perfumes
2.	Methyl heptenone	$C_8H_{14}O$	126.21	173.11°	1.4434 (n_D^{14})	d^{15}- .8656	Adjunct in the scenting of soaps.
3.	Limonene	$C_{10}H_{16}$	136.23	d-175.5° - 176° 1-175.5° - 176°	d-1.474 1-1.474	d^{21}-1.8402+ 0.8407	Wetting and dispersing agent.
4.	Dipentene	$C_{10}H_{16}$	136.23	177.6°	1.4744	..	-do-
5.	Methyl heptenol	$C_8H_{16}O$	128.21	178-180	1.4495	d^{15}-.8581	
6.	Linalool	$C_{10}H_{18}O$	154.24	198.23°	1-1.4604 d-1.4673	d^{20}-0.8622 -0.8733	In soaps and detergents, in perfumery.
7.	Citronellal	$C_{10}H_{18}O$	154.24	208°	1.446	0.848 - 0.856	+In soap perfume and insect repellent.

Table V contd.

	Name	Formula	Molecular weight	B.P./760 mm	Refractive index	Density	Uses
8.	n-decyl-aldehyde	$C_{10}H_{20}O$	156.26	208.25°	1.4287	d^{20}-0.8502	..
9.	Citronellol	$C_{10}H_{20}O$	156,26	224.42°	..	d - .885 (d^{20}) 1 - 1.4576 (d^{18})	Inperfumery
10.	Nerol	$C_{10}H_{18}O$	154.24	227°	1.462	d^{15}-.8813	Manufacture of perfumes
11.	Citral	$C_{10}H_{16}O$	152.24	228°	1.486	d^{20}-.8888 (a) -.8889 (b)	In the synthesis of Vitamin A.
12.	Geraniol	$C_{10}H_{18}O$	154.24	229.65°	1.4766	d^{20}-0.8894	Inperfumery
13.	Geranyl acetate	$C_{12}H_{22}O_2$	198.24	242°	1.4628	..	Inperfumery
14.	Farnesol	$C_{15}H_{26}O$	222.36	..	1.4877	d^{20}-0.8871	Inperfumery to emphasize floral perfumes

Gildemeister and Hoffmann[14] reported the following properties of East Indian lemongrass oil.

Specific gravity at 15°	0.899 to 0.905
Optical Rotation	+1°25' to 5°0'
Refractive index at 20°	1.483 to 1.488
Aldehyde content by bisulphite method	70 to 85%
Neutral sulphite method	65 to 80%
Solubility	Soluble in 1.5 to 3 Vol. of 70% alcohol

1.9 Yield of lemongrass per hectare

The yield of lemongrass per hectare is small during the first year. It increases during the second year and reaches the maximum during the third and fourth years of the plantation. Thereafter it starts declining progressively. Average yield of grass per hectare per year may be taken as 18 tonnes. When the prices, however are not economical, many of the land owners simply ignore their plantations or rent their lands to small peasants on a share crop basis or for a very small rental. The yields are invariably adversely affected as proper attention is not bestowed on the maintenance of the plantation.

1.10 Quantity produced[8]

As in the case of area under cultivation no authentic information is available regarding quantities

of lemongrass oil produced from year to year. Estimated production of lemongrass oil during the course of last forty years has been worked out from the annual exports and direct enquiries from the trade.

1.11 Distillation[7,8,15]

Until about 40 years ago it was chiefly the wild growing grass which was used for distillation of lemongrass oil, because at that time buyers abroad did not insist on a high citral content for the oil. However now conditions have changed. Today it would be too much costly to collect wild growing grass scattered over wide areas and to separate it from other admixed grasses; the presence of which would yield oil of low citral content. For these reasons only cultivated lemongrass is at present used for distillation.

Crude distillation units are located in the plantations because of the difficult nature of the terrain since the stills are not easily accessible. They are scattered over wide areas. Therefore, the number of stills that are in operation are estimated on the basis of the quantity of oil produced in relation to the capacity of the still. It is estimated

that there are about 4,000 stills in use out of which about 500 are in Karnataka; 300 in Tamilnadu and the rest in Kerala.

Because of the scattered plantations, small holdings and the need for distilling off the oil before the grass wilts, there are no centrally situated large scale modern distilleries. Distillation of lemongrass is carried out only on cottage scale in small, crude, direct fired, indigenous stills. Most cultivators have got their own stills. Others carry their grass to a nearby still for distillation on payment of distillation charges.

The still used for steam distillation of lemongrass oil is a truncated cone placed upon a cylinder which is nearby 1.35 m high and 0.7 m diameter. The cone has a vertical height of 0.3 to 0.5 m. In olden days, the entire still was made of copper. With the increasing cost of copper, alternate material had to be found to make the distillation process as economical as possible. The cylinder is a mild steel drum of 200 l capacity and the truncated cone and the goose-neck are made of galvanised iron sheet. The capacity of the still is about 250 l. On one side at a height of about 0.6 m from the bottom of the cylinder is a manhole of approximately 0.5 m diameter for

charging and discharging the plant material. The retort up to the manhole is embedded in a hearth made of bricks and mud and is used as a furnace. The mixture of oil vapour and steam passes into a spiral condenser which is made of galvanised iron tube of 2.5 cm diameter fixed to the cone and finally into the receiver where the condensate is collected. The condenser remains immersed in an open tall wooden tub filled with cold water lifted from a well or nearby brook. The tub is about 1.8 m high. It has an opening at the side near the bottom for drawing off warm water during the distillation. The receiver is a squat tin vessel which serves as a Florentine flask for oil separations. The still is set up in a wide thatched shed to protect workers from rain and sun, in the vicinity of a brook or a well.

A charge of 300-350 bundles of freshly cut grass weighing 90-100 kg is packed tightly into the still through the manhole. Fifty litres of warmwater from the wooden tub are poured into the still to fill it up to about ¼ of the height. The hearth is lighted with firewood and direct heat applied to the still. The grass gets compressed as the temperature of the water increases enabling some more bundles of

grass being packed into the still through the manhole. The manhole is closed with a lid provided with a handle. The lid is plastered with mud to prevent escape of steam. Distillation starts in about 20 to 30 minutes and is continued till the distillate is free from oily layer. As a good part of the green grass remains soaked in boiling water throughout the distillation, the operation in the still follows the principle of water or hydro distillation.

The vapours are condensed in the spiral condenser immersed in cold water and the mixture of water and the essential oil collects in the receiver. The oil floats on the top. The separated water is drawn continuously through a side opening at the bottom. The oil is carefully skimmed off with a ladle and poured into glass bottles. Condensed water is discarded and not returned to the still for the distillation.

The still is discharged by removing the spent grass by means of a long stick with iron hooks on one end. The spent grass is sometimes used for fuel purposes and it is also a valuable mannure.

Distillation of one charge takes two to two and a half hours yielding 300-400 gms by weight of oil. This means an yield of 0.3 to 0.5% calculating on the

maximum charge of 90 - 100 kgs of grass. With the age of the plantation the yield of oil may increase to above 0.5% but in that case the output of grass per hectare decreases. During the peak season, 4 to 5 distillations are done per still per day. One labourer can manage the still.

The cost of an average size distillation unit is approximately Rs.2,000/- including the installation charges. The truncated cone and the goose neck made of galvanised iron sheet are to be replaced after one year. The entire still is to be replaced after 2 years. The condenser unit lasts upto 10 years.

The solubility of the oils, however, was affected by drying of the leaves. The solubility of oils distilled from dried grass decreased more rapidly on aging than the solubility of oils distilled from fresh material. Experiments carried out by the Puerto Rico Experiment station, United States Department of Agriculture[16], led to conclusions significant in field management. It was found that when grass is dried to 45 to 66% of its original weight, it nevertheless yields almost the same amount of oil having the same content of citral as fresh grass; but the drying results in a great saving of field labour. The procedure would also affect an

economy in the fuel required for distillation. A comparative study of the yield and quality of the oil from dried and fresh grass is given in Table - VI.

As was to be expected, exposure to sunlight progressively reduced the yield of oil for the first three days on the basis of both the fresh and the treated leaves. However on the fourth day there was a surprising increase in the percentage of citral content of the oil which was still further increased on the basis of the treated grass on the fifth day.

1.12 Planting Cultivation and Harvesting[17]

In Guatemala the lemongrass plant (West Indian type) never flowers. Propagation, therefore, has to be effected by root division. The root of a full grown plant yields ten segments. For replanting, each segment is cut to a length of about 2 ft. At the beginning of the rainy season (May), the segments are planted 3 ft. apart, in rows of 3 ft. apart. Three to four segments are planted into each hole, which should be about 6 inch deep. Deeper planting is dangerous because the plants may develop root rot during the rainy season.

The leaves can be cut for the first time eight months after planting, and then every 3 months,

Table - VI

Period of Drying in sunlight	Moisture content of grass in percent	Yield of oil calculated on weight of fresh grass in percent	Specific gravity of oil	Citral content of oil in percent
Distilled immediately	80.27	0.348	0.8945	77.50
One day's drying	79.69	0.345	0.8900	76.60
Two day's drying	77.82	0.287	0.8924	73.00
Three day's drying	71.22	0.244	0.9001	73.04
Four day's drying	66.50	0.344	0.8970	77.84
Five day's drying	43.50	0.366	0.8954	79.00

which permits four annual harvests. Except for weeding and cleaning of the fields, not much work is necessary. The plants are cut by hand a practice involving a great deal of labour contributing maximum towards the total cost of the oil. After 4 years, the productivity of a lemongrass field declines to such a point that the planting should have to be renewed.

Recently, machinized methods of farming have been introduced on certain large estates devoted to the cultivation of lemongrass and citronella. Distilling equipment also has been modernised.

1.13 Yield of Oil

The yield of oil from lemongrass varies with the area of cultivation, rainfall, type of soil, the strain of the grass and the attention paid to the field with regard to weeding, manuring etc. Lemongrass being a perennial plant, the yield of the oil varies with the age of plantation. On an average, the yield of oil in lemongrass varies from 0.3 to 0.5 percent. The life of a plantation of lemongrass varies from 4 to 6 years depending mainly upon the type of soil. As in the case of grass, it is observed that the yield of the oil per hectare is less in the first year. It starts increasing in the second year and reaches its

maximum in the 3rd and 4th years. It decreases after that. On an average it is estimated that the yield of oil per hectare per year is 60 kgs.

According to the information given[17] by the Oficina Controlodora de Aeites Esenciales, Guatemala, C.A. in commercial production, the yield of oil per acre per year declines with the age of a field. Figures furnished by the Oficina in 1950 indicate that, with 3 or 4 cuttings per year, annual yield of oil per acre (in large scale production) was 96 lb. in the first year, 74 lb. in the 2nd year, 66 lb in the 3rd, and 48 lb in the 4th year. Average yield of oil per acre per year was thus 71 lb.

In experiments conducted by Loustalot and Pol[18], East Indian Lemongrass outyielded West Indian in terms of fresh grass produced. However the yield of oil per acre was greater from West Indian grass when cut at maximum height and at low weight because the percentage of oil from the grass was higher. East Indian grass cut at the same time and height, because the percentage of oil was about the same and the East Indian variety yielded more grass. The average annual yield of oil per acre from plots of West Indian grass cut ten, seventeen and nineteen times over a three year period was 131.0 lb, 132.3 lb and 110.5 lb respectively. The

average annual yield of oil per acre from East Indian grass harvested at a height of 2.5 ft. was at least 100 lb more than when the grass was cut maximum or at low height. The percentage oil was consistently higher in the West Indian grass, but there was no marked difference in citral content between the two varieties or among the 3 harvests.

1.14 Handling and Marketing of the Oil[9,14]

The distillers in India collect crude oil in bottles. At the time of collection it still contains small quantities of water, suspended impurities and has a turbid appearance. The oil has to pass through the hands of several field brokers, intermediaries and dealers before it r aches the godowns of the exporters. Standard lots of oil containing at least 75% citral are made up and shipped abroad in galvanised iron drums. The best time for purchasing the oil in Cochin for shipment is at the end of the main producing season (November to January). Great quantities are then available at low prices. Immediately after the producing season the oil has the highest citral content which is another reason why oil should be shipped at this period. Buyers abroad insist upon oils of most recent harvest.

1.15 Utilisation

1.15.1 Exports of oil[9]

Lemongrass oil exports from India were insignificant until the beginning of the present centuary. An export market came into existence towards 1903-04. Temptation to adulterate oil, however, caused the price to fall below the economic level and exports again went down. Oil was shipped both from Cochin and Alleppey. During the World War I, the volume of trade decreased because of shipping difficulties but again started increasing steadily after 1918 - 1919. The production of the oil during these years outstripped demand and there was a large carry over. After the World War II the demand suddenly increased. In the five years preceeding World War II, India exported the following quantities of lemongrass oil[19].

	Hundredweight
1934	6,062
1935	7,067
1936	6,956
1937	7,146
1938	7,694

During and right after the war the figures fluctuated considerably, as can be seen from these statistics[20].

Financial year (ending March 31st)	Quantity of oil in Gallons
1939 - 1940	100,135
1940 - 1941	83,535
1941 - 1942	122,924
1942 - 1943	59,452
1943 - 1944	54,306
1944 - 1945	121,629
1945 - 1946	150,790

According to Volkart Brothers, Inc.[21], New York, the exports of East Indian Lemongrass oil (in longtons) from 1942 to 1948 are as follows.

1942 - 1943	114
1943 - 1944	382
1944 - 1945	566
1945 - 1946	505
1946 - 1947	465
1947 - 1948	232

During the 1947-1948 season, the united states took 85 tonnes or 38.33% and United Kingdom 52 tons or 23.64% of the total exports.

A slump followed with low prices in 1950 when the United States of America started stockpilling of raw materials due to outbreak of war in Korea. By this time the manufacture of synthetic Vitamin A from

citral, the major constituent of lemongrass oil, had started on a commercial basis. Although it was expected that demand for this indegeneous industry would building up swiftly it did not do so till 1965-66. All these years the export figures were at high levels and it was only in the year 1966-67 that the exports came down to as low a level as 46% of total production in India. Since then the exports have been declining and was even less than 10% in 1978-79 (71.6 tonnes). However, the exports improved to account for about 37% of the total production of about 650 tonnes during 1979-'80.

Over the last two decades there has been a total change in the pattern of exports. There was a time when 97% of lemongrass oil produced in India was exported and India was meeting 80% of the World demand for this oil. Today only about 15 to 30% of the total production is exported. The rest is consumed within the country.

Exports have dwindled considerably during the last 2 decades. It has come down from 1258 tonnes in the year 1962-63 to mere 71.6 tonnes in the year 1978-79. However it improved to 240.9 tonnes during the year 1979-80. This marked decline in exports is

to be attributed to the increased demand within the country for this oil and the shrinkage in the area under cultivation of lemongrass.

The oil for export is packed in 200 l mild steel drums. These drums are used in fact as second hand lubricating oil drums in sound condition throughly cleaned and painted. Although the oil packed in these drums is absolutely clear by prolonged settling or by centrifugal clarification, due to the corrosive action of the oil on the steel, some sediment is found by the time the drums reach the destination. Longer the storage of the oil in these drums, greater is the sediment in the drums. However the sediment is not so much as to elicit any complaint from the importers. Due to the lapse of time the citral content would have slightly dininished by the time the oil reaches the destination but this has been taken care of by fixing the minimum citral content at 76% for export purpose, whereas the contracts are on the basis of 75% citral content.

All the exports of lemongrass oil are chanalised through the State Trading Corporation from 30th May,1964.

In the fifties, U.S.A., U.K., France, Netherlands, Switzerland and Germany followed by other countries were the importing countries in order of importance. The position has totally changed since then. The USSR has emerged as the biggest buyer followed by U.K., U.S.A. and other countries.

1.15.2 Internal Consumption

There was a time around year 1955-'56 when 97% of the total production of lemongrass oil in India was being exported and hardly 20-25 tonnes were utilised within the country mainly in the soap industry and to a very small extent in the manufacture of ionones for perfumery. The manufacture of Vitamin A had not yet commenced. Since then, utilisation of lemongrass oil within the country has increased steadily and today about 70 to 90% is utilised within the country. The approximate total requirement of lemongrass oil for the internal consumption in our country has been estimated as follows:

a) Aromatic chemicals/perfumery compounds about 250 tonnes per annum.
b) Soap and toiletaries 150 tonnes per annum.
c) Agarbati and other miscellaneous uses 50 tonnes per annum.

This consumption of 450 tonnes is also likely to increase in future in the proportion of population growth of the country. Synthetic Vitamin A is being manufactured in India by M/s.Glaxo Laboratories, Bombay, M/s. Rosche Pvt. Ltd., Bombay and Kerala State Drugs and Pharmaceuticals, Alleppey, Kerala. The estimated consumption of lemongrass oil, quantity of end products and their values in 1982 has been given by M/s.Glaxo Laboratories, Bombay is given in Table - VII.

Most of the quantity of Vitamin A manufactured in India is being utilised in the country. The β-ionone is exported to U.S.S.R. and some other countries of the world where it is utilised for the manufacture of Vitamin A and perfumes. β-ionone is also used in our country for manufacturing perfumes but in a very small quantity.

It is pertinent to note that India exports its lemongrass oil and β-ionone to other countries while their end products i.e. Vitamin A and perfumes are imported back to our country. The demand for lemongrass oil internationally has declined due to synthetic methods of ionone manufacture. It is also stated that the cost of ionone produced in India is more than that of synthetic ionone produced aborad. But from the data given by M/s.Glaxo Laboratories, Bombay, it can be

Table - VII

Year	Input	End products obtained			
	Quantity of lemon-grass oil used.	Vitamin A		β-ionone	
		Qty. (M.T.)	Value (Rs. lakhs)	Qty. (tonnes)	Value (Rs. lakhs)
1974-75	160	8.1	46	103	156
1975-76	180	11.0	62	111	168
1976-77	161	11.0	62	92	139
1977-78	188	16.8	94	106	161
1978-79	194	21.2	119	109	165

seen that there is a significant difference in the value of lemongrass oil and its finished products. Therefore there is a need to explore the possibilities for the utilisation of lemongrass oil to manufacture finished products and export them to other countries. India will earn more foreign exchange from these finished products i.e. Vitamin A, β-ionone and perfumes.

The perfumery, soap and detergent industries have also expanded enormously and the consumption of lemongrass oil by these industries has considerably increased. After meeting the requirements of our industries, whatever is left over is exported to other countries. This quantity accounts for about 15 to 30% of our total production. The exports have come down considerably. But at present the matter of great concern is that our production of lemongrass oil which was at a peak of 1700 metric tonnes in the year 1963 - '64 has also come down to 400 metric tonnes now. In this span of time there has been considerable industrial developments. A trend of exporting finished products has developed in India, and now it has started its export of finished products to other countries and the time is not far off when the quantity of lemongrass oil which we are exporting to other countries shall also be consumed in our own country and

there would not be any oil left over for export. The cause of great concern here, is that when we started our production of finished products for export purposes, the production of raw material i.e. lemongrass oil is declining fast and if the production is allowed to decline further, it may result in a situation of scarcity of raw materials to our lemongrass based industries and also cause rural unemployment. This trend of declining production is not a good sign especially when the production of finished products has come within the grip of our industries. There is a need to extend its production further to have the raw material at a lower cost so that Indian products can be competitive in the international market.

1.16 Wholesale prices

It may be seen from these data that wholesale prices continuously decreased from 1965 to 1969 which could be attributed to the decline in the production of lemongrass oil in the country. In 1970-'71 a sharp decline in price was noticed due to demand in the foreign markets coupled with increased production of the oil. Thereafter, it marks the transition period from an export oriented trade to increased internal consumption. The exports declined steeply and the consumption of the oil within the country increased

rapidly, with decline in exports, the element of speculation disappeared and the internal demand for the oil maintained the prices high and steady.

1.17 Seasonal Variations

The main marketing season for lemongrass oil is July to January. A seasonal variation in the price of lemongrass oil has been observed.

1.18 Economics of Lemongrass

1. Gives an essential oil	:	Lemonscented oil used in soaps, perfumes etc, contains an aldehyde - citral which can be converted onones and Vitamin A.
2. The exhaust grass from distillation	:	A good nutritive ingredient in cattle feed when added to molasses - residue from sugarcane - for compost making, for straw board and paper manufacture.

3. Gives a net profit of Rs.30/- to 40/- for 1 kg of the oil. Price of 1 kg of the oil is about Rs.140/-.

This also logically explains why the settlers continue to plant lemongrass inspite of the fact that it exhausts the soil to a considerable degree. The plant takes nourishment, but it is fed back to cattle in the form of grass who return it to nature thus achieving the circulation of organic matter in nature.

During past several years, improved strains of lemongrass have been established and are under exploitation in various parts of the country. In the last few years CIMAP has tried to introduce cultivation of lemongrass in other states besides Kerala on scientific lines and has given necessary help to farmers for proper distillation of oil, so as to get a good quality lemongrass oil, which could be easily exported. Experiments have been done at CIMAP regional centre, Bangalore in order to develop improved agrotechnology and also to isolate an improved strain with high oil content and high amount of citral. As a result of these efforts certain amount of lemongrass oil is produced in Karnataka, Uttar Pradesh, Assam and Meghalaya[22].

The 3 commercial lemongrass strains RRL - 16 (Jammu), O.D. - 19 (ICAR selection from lemongrass Research Centre, Odakkali, Kerala) and S.D. - 68 were

put on comparison trials in a statistically designed experiment in March 1975 under the climatic conditions of Jammu. The yield of the herb as well as the oil is 60% more from Jammu lemongrass (RRL - 16) compared to O.D. - 19. S.D. - 68 has a marginal lead over O.D. - 19 in its yield in the first year, however it could not maintain the lead in the 2nd year, but in the 3rd year of harvest it again showed a marginal lead in its total oil yield. Jammu lemongrass gave almost double or more than double the oil yields as compared to other two strains in all the 3 years. The citral content as determined by GLC analysis in all the 3 strains was upto B.P. standard (70 to 85%). Jammu lemongrass is thus recommended for cultivation in North India[23].

The comparative efficiency of RRL - 16 with O.D. - 19 was studied at Odakkali Research Station, Kerala and the result obtained showed that it is inferior to O.D. - 19 in respect of total grass and oil production under Odakkali conditions[9].

Other varieties of lemongrass (Cymbopogon flexuosus), has also been reported in India. They are called RRL - 54, collected from Chandi hill and RRL - 59 from Kolar[24].

The chemical constituents of O.D. - 19 and S.D. - 68 have been studied and reported as below[25].

O.D. - 19 has been cultivated at Kukrail Research Farm Lucknow. Samples of O.D. - 19 were distilled by hydro-distillation and the essential oil was obtained in yields of 0.43% on the fresh weight basis. S.D. 68 has also been cultivated at Kukrail, Lucknow and distilled by hydro-distillation. The yield of oil was 0.49%, on the fresh weight basis. The chemical constituents of the 2 varieties which are important from the perfumery point of view are reported below in Table - VIII. In the oil of these varieties Limonene, which is usually common in Cymbopogon species, was found absent.

Performance of S.D. - 68 at Sepahigala and Tripura were studied by Shah and Coworkers[26]. The high yielding Lemongrass Strain (S.D. - 68) was harvested at intervals. Three cuttings per year yielded the highest quantity of herb and oil. The oil contained citral a (52%) and citral b (37.2%). Harvesting at a gap of 180 days gave the highest oil yield.

Table - VIII

Chemical constituents	Percentage	
	O.D.-19	S.D.-68
Myrcene	0.02	0.02
p-Cymene	0.4	0.04
Methyl heptenone	..	0.07
n-decyl aldehyde	..	0.35
Citronellal	..	0.7
Borneol	1.9	0.95
Terpenyl acetate	0.90	1.20
α-Terpineol	2.25	0.38
β-Terpineol	0.40	0.17
Terpinen-4-ol	0.60	0.41
Geraniol and Nerol	1.50	0.78
Geranial	46.60	45.70
Neral	27.70	34.90
Farnesol	12.8	6.00
Farnesal	3.0	2.40
Nerolidol	..	traces

441 types of lemongrass (global collections) were collected in the Lemongrass Oil Research station Odakkali, Kerala for studying the relative merits under the agroclimatic conditions of Kerala and to select suitable types and also for utilising as parent materials in hybridisation work. This is claimed to be largest collection in the world[9].

The lemongrass cultivation is declining nowadays. It is very difficult to obtain the oil because of fuel cost and scarcity of fuel. The standard of living of people have gone up and the high wage has also become a problem. The extraction of oil from the grass is a very labourious work and yet the yield of oil is very poor (0.4%). Another reason for the fall in lemongrass cultivation is the unattractive price of lemongrass oil (about Rs.140/- per kg).

Cultivation of Palmarosa is said to fetch the farmer double the income than lemongrass cultivation and because of this the estimated area under cultivation has come down from 8,000 hectares to 4,000 hectares today and consequently the oil production has come down from the peak figure 1700 tonnes in 1963-1964 to a rather stabilised level of 650 tonnes in 1980-'81.

1.19 Grading of Lemongrass oil under Agmark[9]

Central Government prohibited with effect from 1-1-1956, the export of East Indian Lemongrass oil (Cochin Lemongrass oil) produced in India, unless they are graded in accordance with the provisions of the Essential oils grading and marketing rules of 1959, promulated under the provisions of the Agricultural produce (Grading and Marketing) Act, 1937.

The Indian Standards Institution in consultation with the essential oils committee of the Council of Scientific and Industrial Research had drawn up standards for lemongrass oil (IS 327-1952) in 1952. It was decided to have 2 grades of lemongrass oil, i.e. "Spe ial" grade with percentage of citral not less than 80 and "Grade A" with percentage of citral not less than 76. Specifications for other factors of purity and quality (specific gravity, refractive index, optical rotation, solubility in 70% alcohol) are the same in both grades.

Details regarding the day today working of the scheme are contained in the "Instructions for grading lemongrass and Sandal wood oils" issued by the Agricultural Marketing Adviser to the Government of India in 1955. These have been drawn up in consultation

with the trade interests. Some of the important features of the grading scheme are discussed below:

Compulsory grading of lemongrass oil and pre-shipment inspection of the graded oil has come into force on 1-1-1956.

Any packer, after getting certificate of authorisation from the Agricultural Marketing Adviser to the Government of India, can apply for grading of the oil, to the Deputy Senior Marketing Officer - Essential oils, grading scheme, Willington Island, Cochin-3, to inspect and sample the oil. There upon an inspecting officer inspects the premises, analyses each lot and seals with special code marks, after checking the soundness and cleanliness of the containers. After that these samples are submitted to analysis at the Regional Agmark Laboratory at Willington Island under code marks. The analysis will be completed within 48 hours of their receipt in the laboratory. Agmark officials then put lead seals on the containers and affix Agmark label and the container are separately marked with the name of the packing place and date of packing, lot number and grade of oil. A certificate is issued to the packer indicating the lot number and grade of oil which has to be produced by the exporter for inspection to the customs.

As the citral content of the oil goes down progressively on storage, the certificate of grading becomes invalid after 45 days from the date of inspection of the oil. Grading of the oil has resulted in a very marked improvement in the quality of the oil. Agmark certificates is done in well established and internationally accepted standards, which enabled the trade to enter into contracts on the basis of description without actually seeing the oil.

1.20 Analysis of Lemongrass Oil

1.20.1 Chemical Composition

Eventhough citral is the main constituent of both West Indian and East Indian Lemongrass Oil, on fractional distillation the two types of oil behave differently[27]. The East Indian oil starts boiling only above 210°C, whereas, at that temperature, 23% of the West Indian Oil has already distilled over. Comparative distillation tests at reduced pressure, and separation of each oil into five equal fractions, showed that the physicochemical properties of the corresponding fractions of the two oils displayed considerable differences, the West Indian type yielded completely inactive fractions only, whereas the fractions of the East Indian Oil had rotations ranging from -12° to -2°. Both types contain 75 - 85%

citral but the West Indian Oil also contains some other aldehydes which are difficult to remove from the citral and may cause trouble in the technical separation and purification of citral[28].

The most complete investigation of the West Indian types of lemongrass oil (origin : Equatorial Africa and Comoro Islands) derived from Cymbopogon citratus Stapf, is that of Naves[29], who noted the presence of the following compounds. Isovaleraldehyde, furfural, myrcene, dipentene, methylheptenone, an aldehyde with molecular formula $C_{10}H_{16}O$ and with Camphor like odour, Citronellal, n-decyl aldehyde, an aldehyde or ketone with an aceptophenone odour, citral-a, citral-b, farnesal, α,β-Dihydropseudoionone, esters like Valerates, Caprates, Citronellates, Geranates and Nerates; Methyl heptenol, l-Linalool, α-Terpineol, Isopulegol, Geraneol, Nerol, citronellol, farnesol, a bicyclic sesquiterpene and α-Camphorene. Naves[30] reported that the oil of lemongrass distilled from Cymbopogon flexuosus stapf in Guatemala contains about 0.7% of a diterpeneketone $C_{20}H_{30}O$ which combines with the reagent of Grignard and Sandlesco under the usual conditions. The following components are characterised in the Indian Lemongrass Oil by Zamureenko et al in 1981 using a

combination of gas chromatography and microspectroscopy[31]. Myrcene (1.5%), Methyl heptenone (0.2%), Linalool (1.2%), Linalyl acetate (0.1%), 2-undecanone (0.3%), Geranyl acetate (0.1%), citronellol,(1.5%) Nerol (0.8%), Neranyl acetate(1.8%), Neral (43.50%) and geranial (28.8%). In 1982 Formarck and Kubeczka characterised the presence of the following compounds in a sample of East Indian Lemongrass oil[37]. ∝-Pinene (0.24%),∝-Thujene (0.03%), Myrcene (0.46%), Limonene (2.42%), cis-β-Ocimene (0.06%), trans-β-ocimene (0.07%), Terpinolene (0.05%), Methyl heptenone (1.43%) 2-nonanone (0.07%), Citronellal(0.37%), Linalool (1.34%), Caryophyllene (0.32%), Neral (30.06%), ∝-Terpineol (0.38%), Geranial (51.19%), Geranyl acetate (1.95%), Citronellol (0.44%), Nerol (0.39% and Geraniol (3.8%).

In 1983 Sarer[33] used liquid solid chromatography G.C. and M.S. to examine the chemical composition of 2 laboratory distilled samples of Turkish grown Cymbopogon citratus and identified the following components.

∝-pinene (trace)

∝-thujene (trace-0.1%)

Myrcene (8.2 - 19.2%)

trans-paramentha-2-ene-1-ol

∝-terpineol(0.2-0.3%)

Limonene (trace)	Neral (25 - 28%)
cis-β-Ocimene (0.2%)	Geranial (45.2 - 55.9%)
1-8-Cineole (0.2%)	Geranylacetate (1%)
trans-β-Ocimene(0.1 - 0.2%)	Nerol (0.3 - 0.4%)
6-Methyl hept-5-ene-2-one (0.5 to 2.3%)	Citronellol (0.1%)
Nonanal (0.2 - 0.7%)	Geranial(0.5 - 0.60)
∝-thujone (0.1%)	Citronellal (0.1%)
Linalool (0.8% - 1.1%)	Undecan-2-one (0.4 - 0.6%)

Abegaz et al (1983) examined the chemical composition a sample of C.citratus oil of Ethiopian origin having using a GC - MS system and characterised the following compounds in the oil[34].

∝-Pinene (0.1%)	Citronellol (0.1%)
Myrcene (0.1%)	Menthone (0.2%)
Δ-3-Carene (0.1%)	Fenchone (0.3%)
Terpinolene (0.1%)	Neral (3.3%)
Ocimene (0.2%)	Geranial (10.2%)
Linalool (3.4%)	Borneol (15%)
Camphor (0.2%)	Menthol (0.5%)
Nerol (4.5%)	Geraniol (40.2%)
∝-oxobisabolene(12.1%)	

In the same year Taskinen et al (1983) used GC - MS to characterise the following components in an oil obtained from Indian grown flexuosus[35]. ∝-pinene, β-pinene, sabinene, myrcene, limonene, p-cymene, 3,7,dimethyl-oct-1-ene, Caryophyllene, ∝-bergamolene, ∝-humulene, ∝-curcumene, β-bisabolene, γ-cadinene, δ-cadinene, 1,8, cineole, 3,7-dimethyl-5-hepten-2-one, perillene, menthone, linalool, linalyl acetate, neral, neryl acetate, piperitone, geranial, nerol, p-cymene 8-ol, geraniol, methyl eugenol, eleminin.

(Neral + Geranial)	=	60%
Geraniol	=	5%
Hydrocarbons	=	8%

1.20.2 Quantitative Determination Of Citral In Lemongrass Oil

The quality of lemongrass oil is determined by its aldehyde content (chiefly citral) which varies from 70-80% Commercial contracts are made on the basis of citral content of the oil. The main methods for determination of citral are

1. Sodium-bisulphite adducting method[36]
2. Neutral sulphite methods[37]
3. Hydroxyl amine method[38]
4. Colorimetric method[39]

Each of these methods have certain disadvantages. Of these bisulphite adducting is the commonly used method for determining the percentage of citral in lemongrass oil. In this method all the aldehydes like citronellal, n-decyl aldehyde etc. and methyl ketones like methyl heptenone known to be present in lemongrass oil get adducted along with citral and hence percentage obtained will be higher than the actual citral content. Disadvantages are found also in the neutral sulphite and hydroxylamine methods. Eventhough the colorimetric method gives more accurate values than the other methods it needs a lot of work and also solutions of citral with known strength. A detailed discussion of these methods and their disadvantages will be given in Chapter IV. A new method has been developed for the estimation of citral in lemongrass oil by physical separation of citral which has none of the disadvantages discussed above and can be taken as an accurate method for determining the correct percentage of citral in lemongrass oil.

1.20.3 Isolation of Citral from Lemongrass Oil

At present citral is isolated from lemongrass oil by sodium bisulphite adducting method[40]. Citral

obtained by this method will not be pure since all other aldehydes and methyl ketones present in lemongrass oil will get adducted together with citral and on decomposition will give a mixture of citral with other aldehydes and methyl ketones present in lemongrass oil. Also there is a possibility of rearrangements taking - place when the adduct is decomposed[41]. For commercial purposes citral is manufactured by vacuum distillation of Lemongrass oil using efficient fractionating columns. By this method citral of about 95% purity is obtained. As an extension of the new column chromatographic method of estimation of citral in lemongrass oil a column chromatographic method has been developed for the separation of pure citral (purity 99+% by GLC) quantitatively.

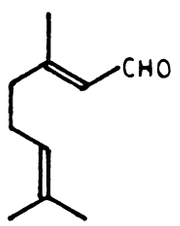
C H A P T E R II

BIOSYNTHESIS OF MONOTERPENOIDS

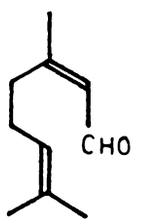
2.1 Biosynthesis of Monoterpenoids

The components present in lemongrass oil are acyclic and cyclic monoterpenoids to the extent of 99%. Hence a discussion on the current ideas of the biosynthesis of these class of compounds will not be out of place.

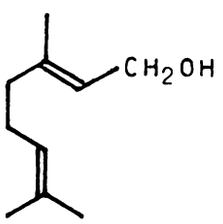
The monoterpenoids reported to be present in lemongrass oil[1] are given below:



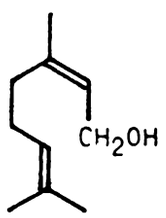
1 Geranial (citral-a)



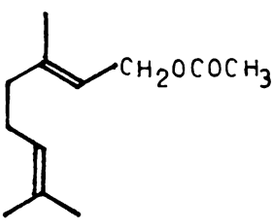
2 Neral (citral-b)



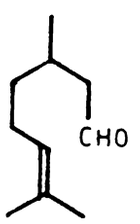
3 Geraniol



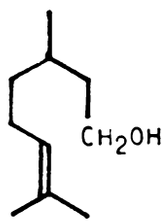
4 Nerol



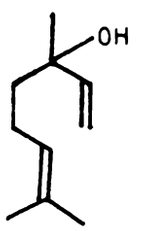
5 Geranyl acetate



6 Citronellal



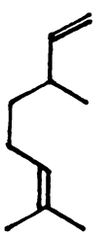
7 Citronellol



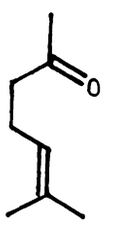
8 Linalool

9 Myrcene

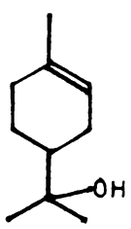
10 Cis-β-Ocimene



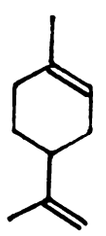
11 trans-β-Ocimene



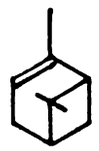
12 Methyl heptenone



13 ∝-Terpineol



14 Limonene

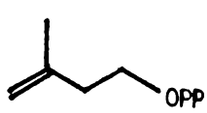


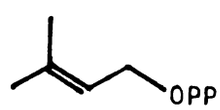
15 ∝-pinene

16 ∝-Thujene

17 Terpinolene

It is by now well established that the biological equivalent of isoprene present in nature is isopentenyl pyrophosphate - IPP (18)[2] and to trigger the biosynthesis of terpenoids the presence of atleast one unit of dimethyl-allyl pyrophosphate - DMAPP (19)[3] is required, which is easily produced by

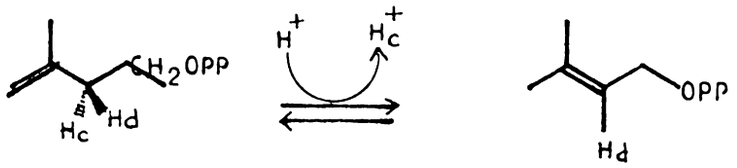




(18) (19)

the enzymatic isomerisation of IPP (Scheme I).

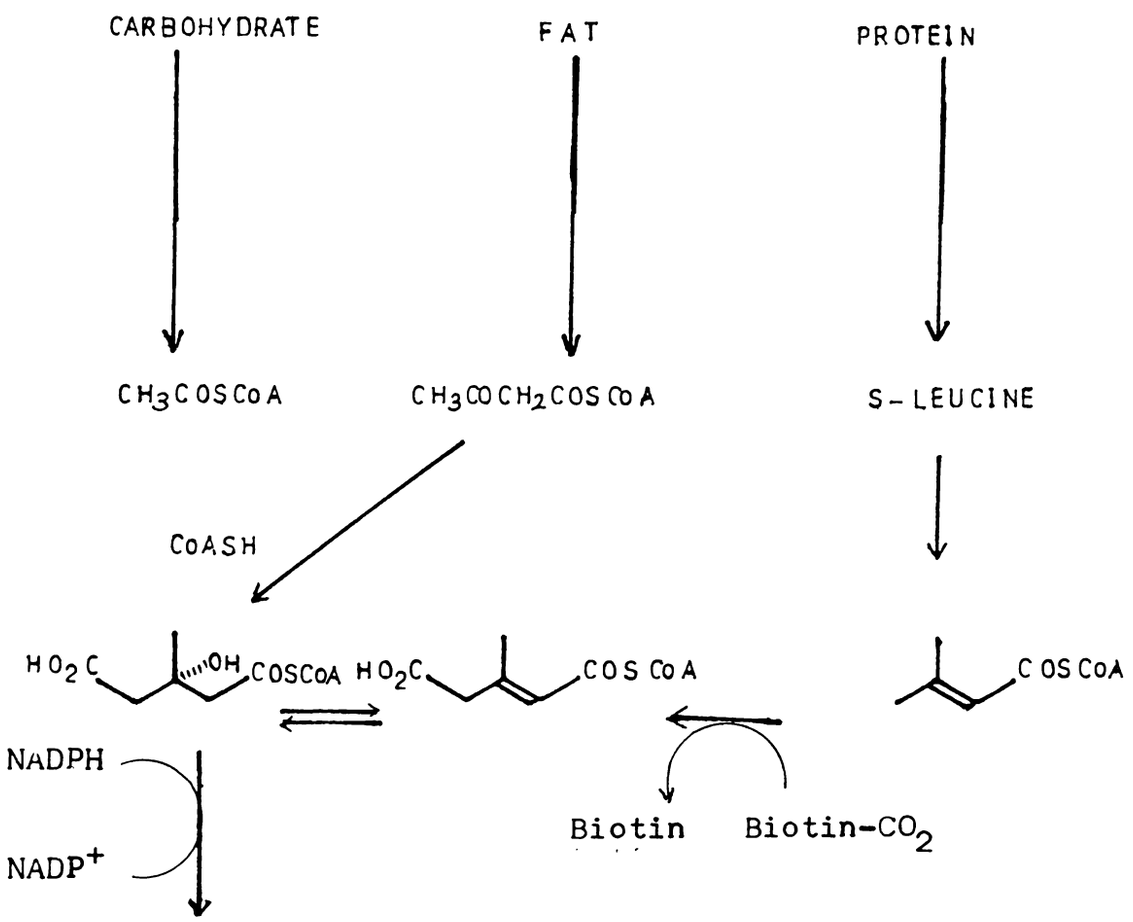
Scheme I

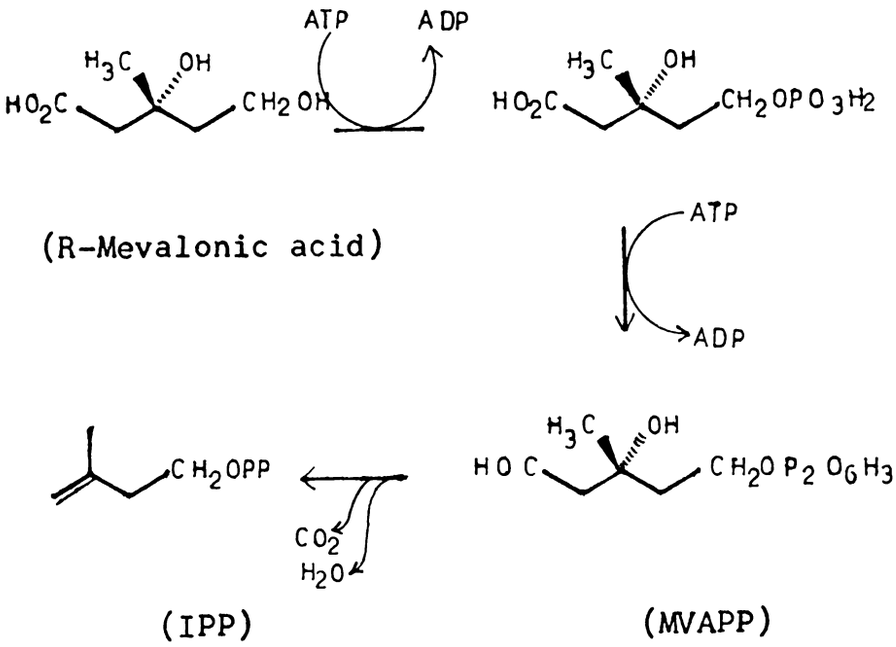


(18) (19)

The current knowledge of the formation of IPP is summarised in Scheme II[2].

Scheme II





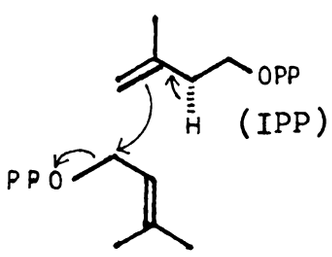
(R-Mevalonic acid)

(IPP)

(MVAPP)

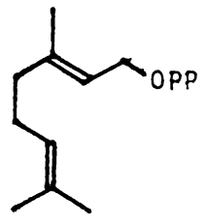
The first monoterpene which is formed by the combination of one unit each of DMAPP and IPP is geranyl pyrophosphate - GPP (20), the mechanism of formation of which is shown in scheme III[3].

Scheme III



(IPP)

(DMAPP)



(20)

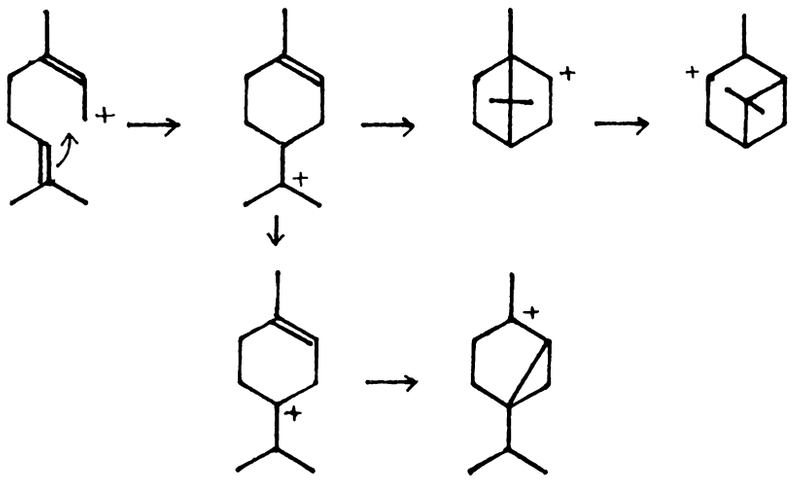
This can be visualised as a case of biological alkylation of the allylic carbonium ion formed by the leaving of the pyrophosphate group from DMAPP, by the attack on the olefinic double bond of the IPP with the concomitent formation of a new trans double bond by the expulsion of a proton. The above alkylation results in the formation of GPP, which is also an allylic pyrophosphate and hence can subsequently undergo similar further alkylation to form higher terpenoids with additional units of IPP in the same manner. Hydrolysis of GPP under mild conditions leads to formation of geraniol. The elimination of pyrophosphate followed by expulsion of a proton and isomerisation can lead to unsaturated hydrocarbons like myrcene (9), Ocimene (10&11)

However it will be difficult to visualise the formation of neryl pyrophosphate - NPP and nerol by the above mechanism.

If GPP is proposed as the main precursor of all monoterpenoids, it is rather difficult to visualise the formation of cyclic (mono and bi) monoterpenoids. The facile formation of cyclic monoterpenoids require as a precursor an allylic carbonium ion with a cis double bond, the formation of which in quantity has to be explained. The facile cyclisation and formation of monocyclic and bicyclic compounds generally encountered can be visualised from such an allylic carbonium ion with a cis double bond

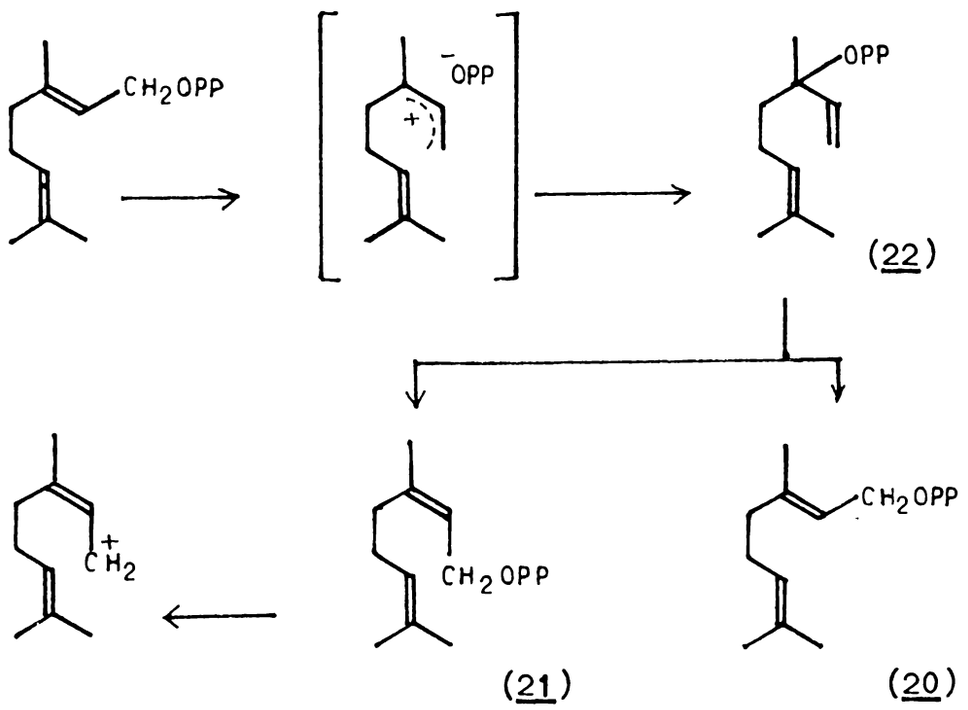
as shown in Scheme IV[4,5].

Scheme IV



Since GPP is efficiently cyclised without formation of free intermediates, it is clear that monoterpene cyclases are capable of catalysing both the required isomerisation and cyclisation reactions, the overall process being irreversible in all cases[6,7]. One possibility was thought to be an enzymatic isomerisation of the trans double bond of GPP into cis double bond giving neryl pyrophosphate - NPP[8](21). However recent findings indicate the intermediatary of linalyl pyrophosphate - LPP (22)[9] for such an isomerisation involving possibly the allylic rearrangement of GPP through the formation of an ion pair (Scheme V).

Scheme V

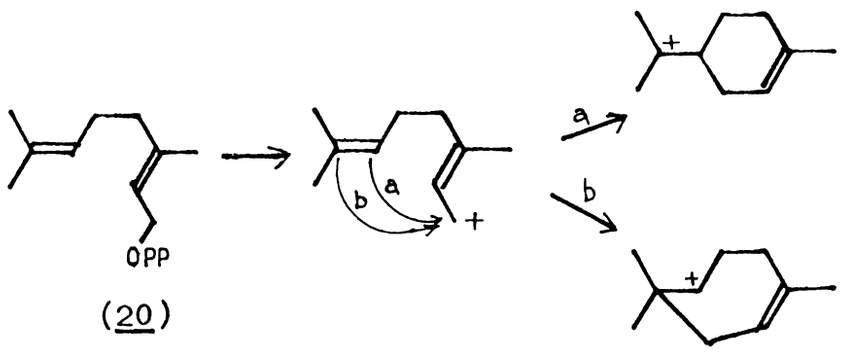


In considering the conformations[10] available for cyclisation of allylic pyrophosphate substrates, the presence of two substituted double bonds in GPP significantly reduce the number of degrees of freedoms of the C_{10} hydrocarbon moiety. Conformations which are capable of cyclisation may be generated by imposing a number of additional constraints on the allylic pyrophosphate substrate.

a) The planes of the two double bond systems of GPP must be perpendicular to a common plane.

b) C-1 must be brought to within the bonding distance of the C_{6-7} double bond (path a and b in Scheme VI) with displacement of the pyrophosphate moiety from C-1 taking place in an antisense.

Scheme VI



(20)

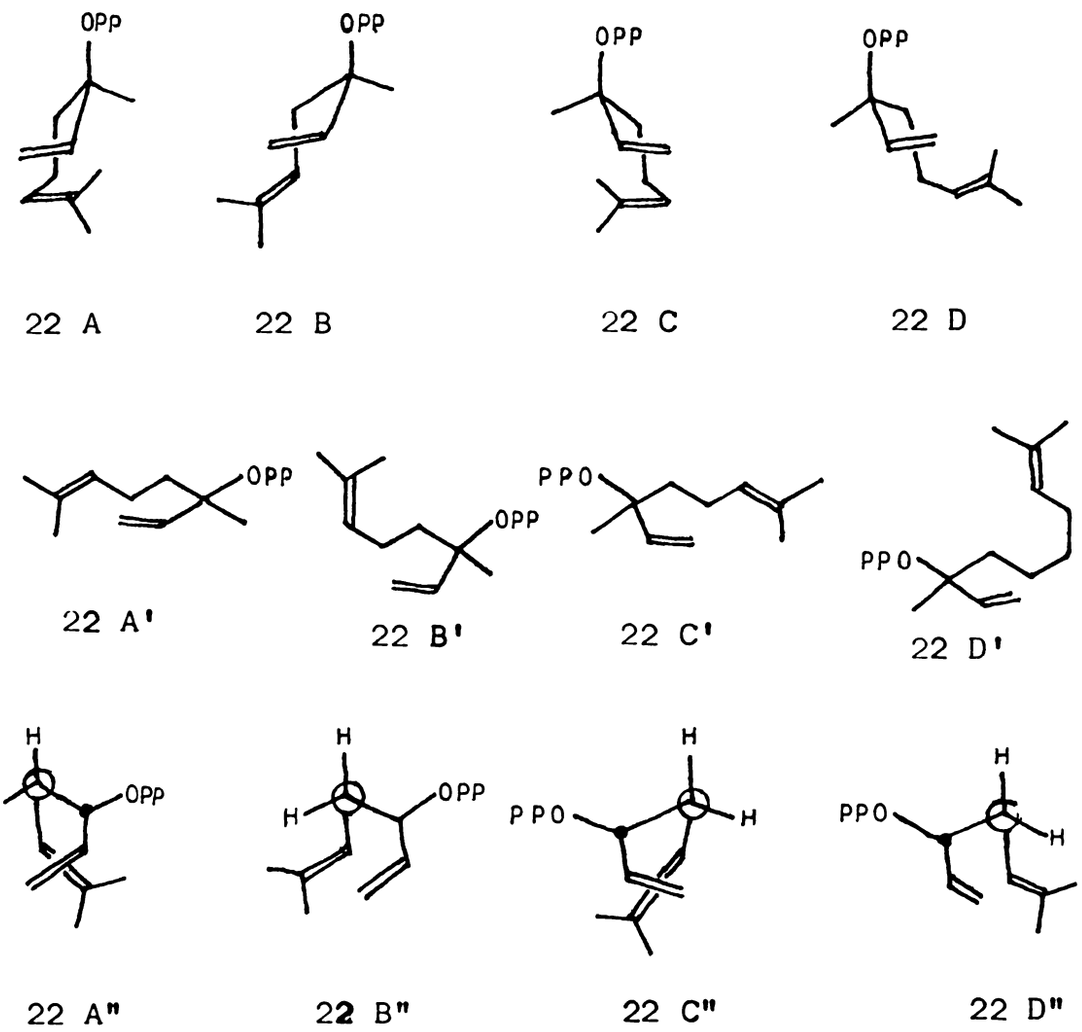
c) For those cases in which a cisoid double bond is generated as a consequence of cyclisation, the cyclisation substrate will be LPP (22).

If the above constraints are imposed, it will be seen that for LPP cyclising by path a and path b , there are only four possible conformations, which can be grouped into two diastereomeric sets of enantiomeric pairs of conformations (Scheme VII).

The pair 22A/22C represent enantiomeric conformations as do the corresponding diastereomeric pair 22B/22D. 22A and 22B have the same configuration (22R) at the single chiral center of LPP but differ as to the face of the 6,7 double bond, syn or anti, respectively, which undergoes

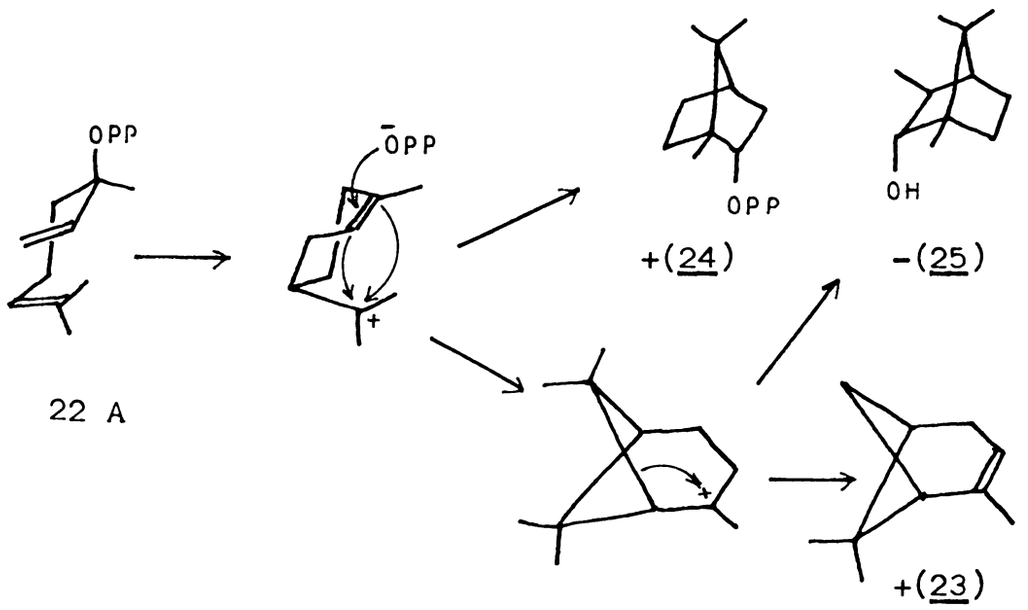
electrophilic attack during cyclisation. For most cyclic monoterpenes, the corresponding conformation of LPP precursor can usually be deduced readily on the basis of the assumption of least motion during the course of cyclisation. There is thus a one to one correspondence between the observed relative and absolute configurations of the terpenoid product and the inferred confirmation of the precursor.

Scheme VII



For example, bicyclic monoterpenes, formed by cyclisation by path a are derived only from conformer 22A and 22C. On the basis of these arguments, it is evident that the bicyclic monoterpenes (+) ∝-pinene(23), (+) -boromyl pyrophosphate (24) and (-) -endofenchol(25) are formed from conformer 22A of (22R)-LPP (Scheme VIII).

Scheme VIII

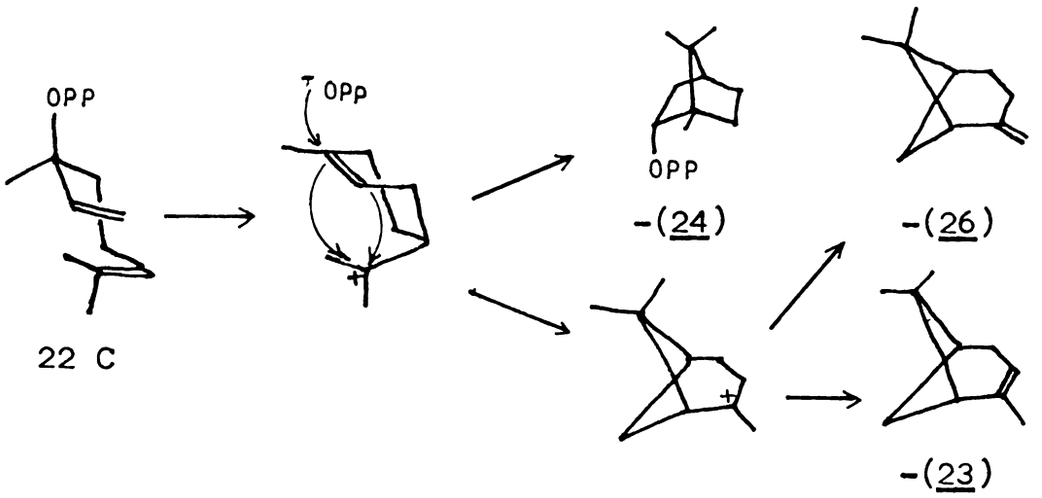


The antipodal series of monoterpenes, including (-)∝-pinene (23), (-)-β-pinene (26) and (-) boronyl pyrophosphate (24) are likewise derivable from (22S) - LPP by way of conformer 22C (Scheme IX).

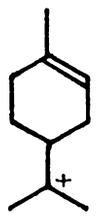
Since all cyclases investigated are reported to utilise GPP as substrate, formation of cyclic products requires initial isomerisation to the tertiary allylic

ester LPP, which is capable of free rotation about the newly generated 2-3 single bond. This isomerisation is believed to take place at the same active site at which the subsequent cyclisation occurs by essentially the same general mechanism, ionisation to form an allylic cation- pyrophosphate-anion pair. The fate of this ion pair depends on its conformation with the transoid conformer undergoing simple recombination at the allylic site (allylic rearrangement), while the cisoid ion pair can suffer backside nucleophilic attack by the neighbouring double bond (cyclisation)[9]. The cyclase must somehow exercise steric conformational and electronic control over the entire cyclisation process. It is reported that (+) and (-) boronyl pyrophosphate synthatases can each cyclise either enantiomer of LPP with only modest enantiomeric discrimination in spite of the demonstrated ability of each cyclase to synthesize enatiomerically pure products from the achiral precursor GPP[11,12].

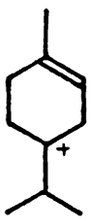
Scheme IX



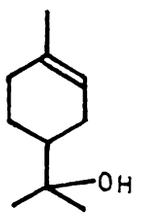
Acyclic monoterpenes undoubtedly arise from chemical modification of GPP, NPP or LPP whereas cyclic members are generally considered to be derived from NPP and LPP. It is not stereochemically possible for GPP to cyclise directly. It is probable that NPP forms a species (27) which can lead to terpineol (13) or can undergo hydride shift to form (28), the progenitor of terpinen-4-ol(29).



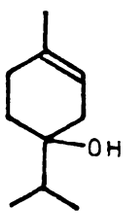
(27)



(28)



(13)

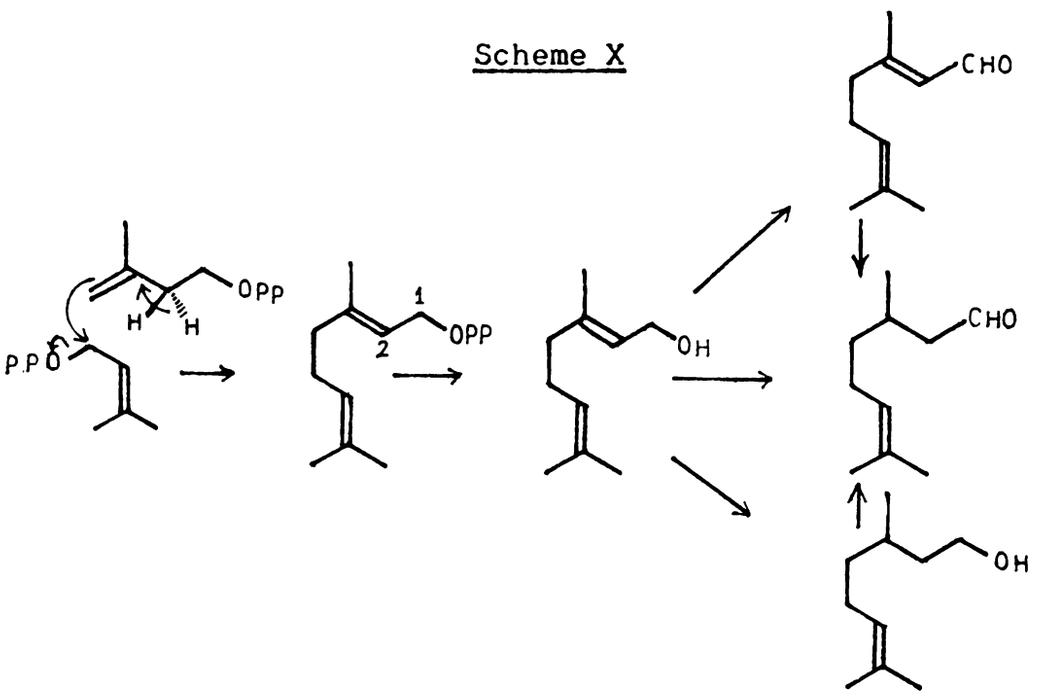


(29)

Dehydration of these alcohols or elimination of a proton from the corresponding carbonium ions leads to menthadienes. Formation of citronellol and citronellal in Cymbopogon winterianus was studied by isotopic labelling technique[13]. It was proved that the 4 S hydrogen from MVA was specifically lost during the condensation of IPP and DMAPP units and established that the 4R hydrogen from MVA remained at

C-2 of geraniol, citronellol and citronellal at all stages of biosynthesis. In the experiment with ($5\text{-}^{3}H_{2}\text{-}2\text{-}^{14}C$) MVA, geraniol and citronellol had the same isotope ratio as the precursor whereas citronellal had half the ratio of the precursor. This showed that one of ^{3}H atoms at position C-1 in geraniol or citronellol had been lost during the oxidation at C-1. Scheme X shows the formation of citronellal.

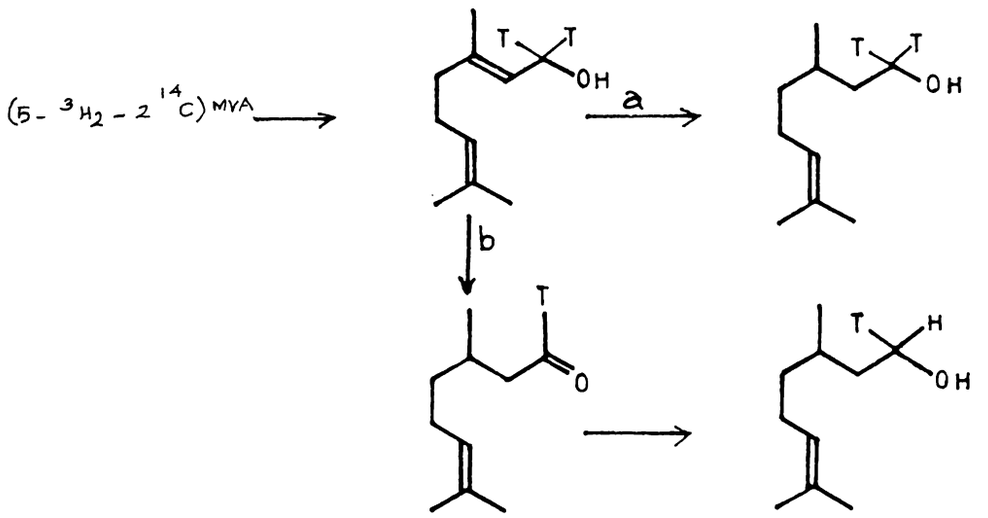
Scheme X



The isotope ratio of the alcohols and aldehydes suggests that there is no active enzyme to convert the aldehyde back to alcohol. The above experiments proved that geraniol is converted to citronellol which in turn is converted to citronellal. There is also a possibility for the conversion of geraniol to citronellal by the

enzyme catalysed double bond rearrangement to give the enol which then spontaneously rearranges to form citronellal since the mechanism would also result in the loss of one ^{3}H atom when citronellal is biosynthesised from (5-$^{3}H_2$-2^{14}C) MVA (Scheme XI).

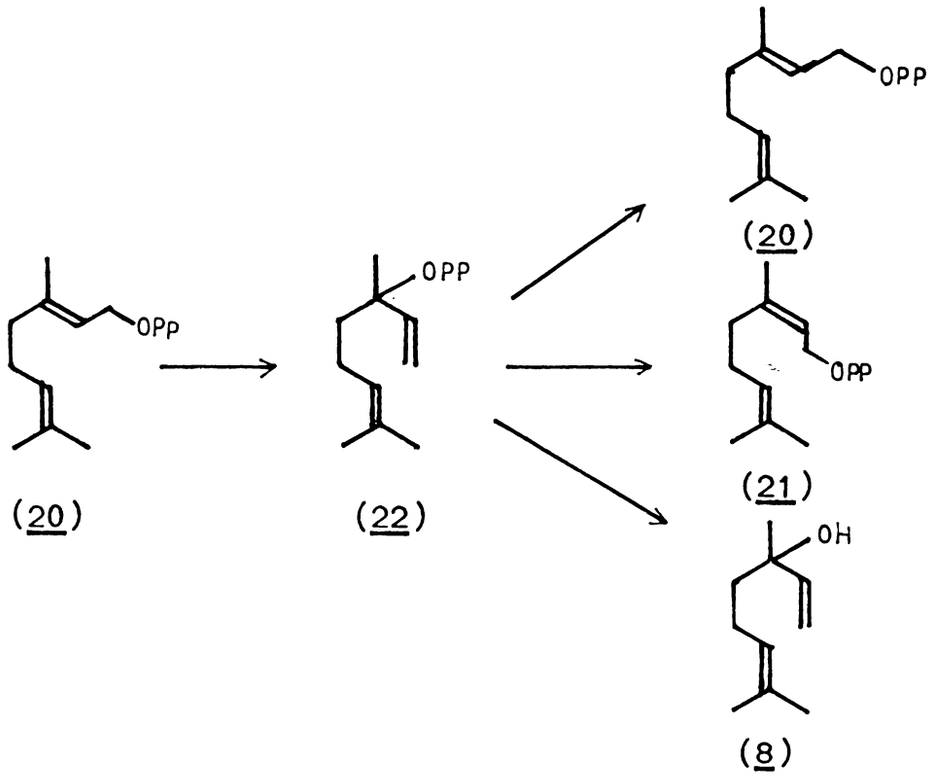
Scheme XI

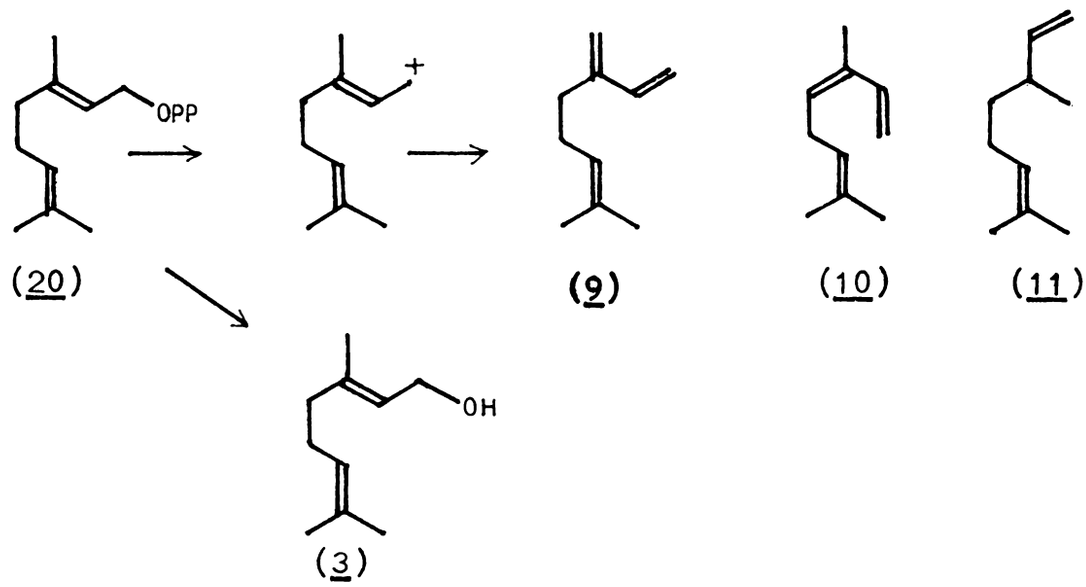


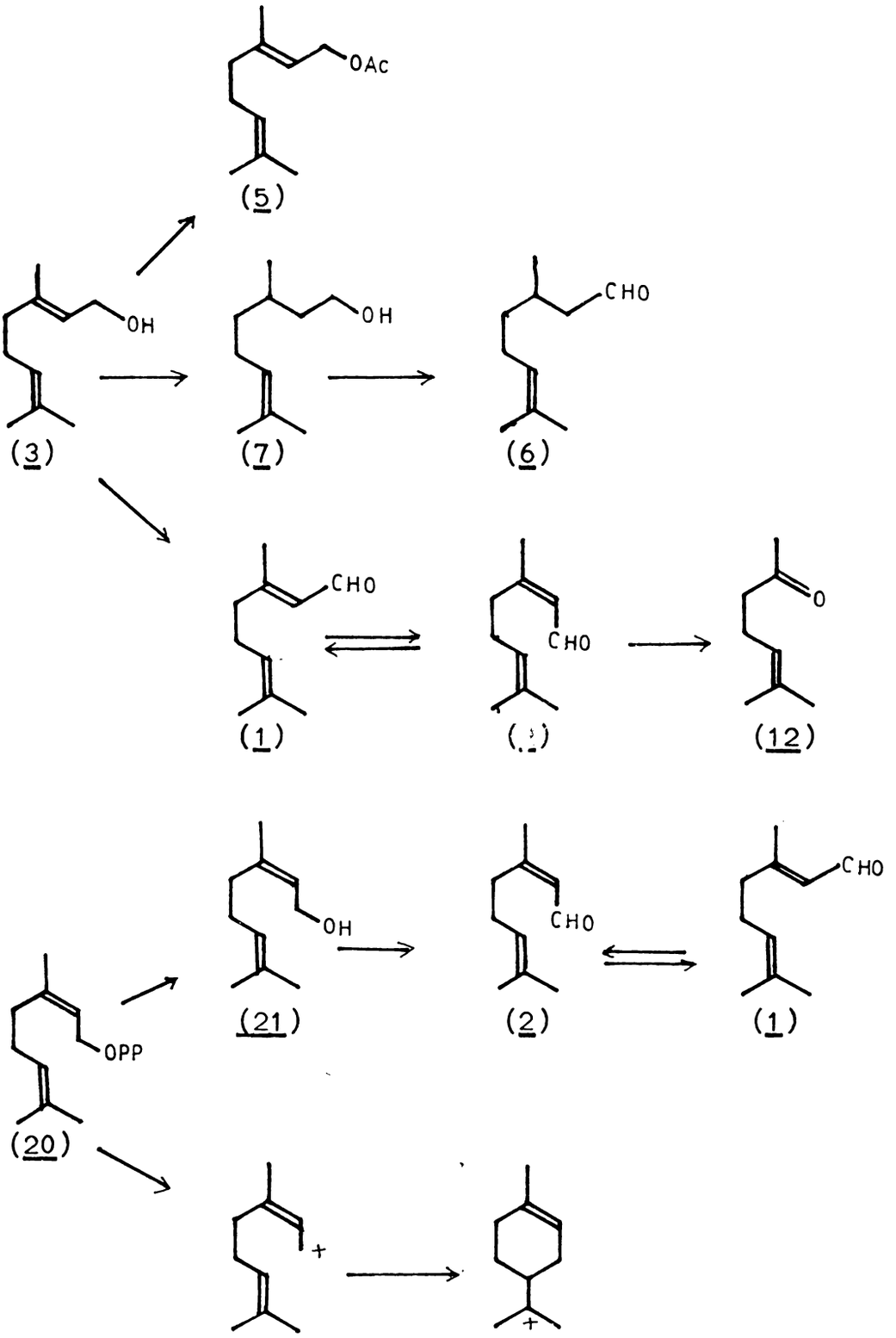
2.2 Biosynthetic pathways of the components in Lemongrass oil

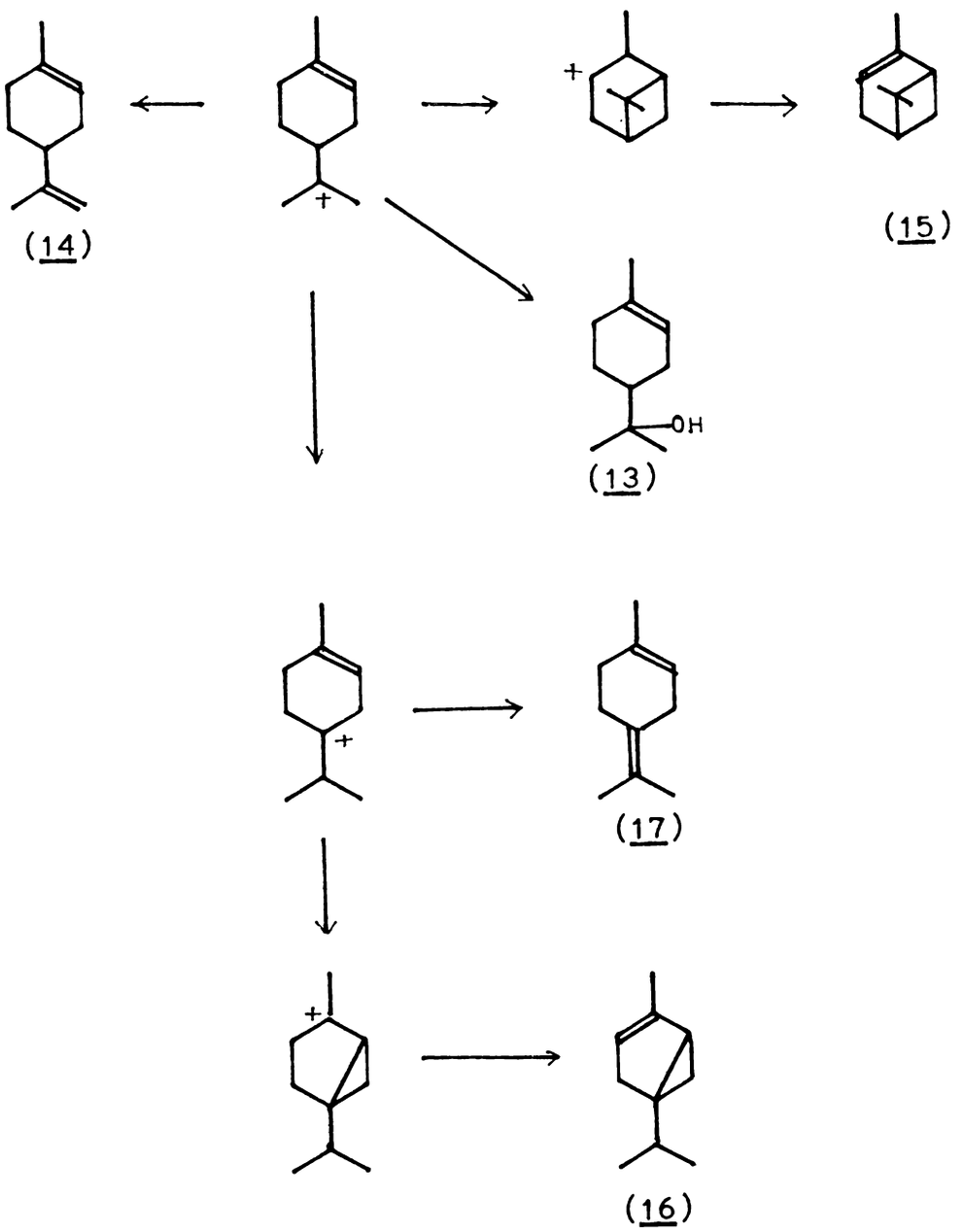
The biosynthetic pathways for the formation of the components present in Lemongrass oil are given in Scheme XII.

Scheme XII






OAc
(5)
OH
(3)
OH
(7)
CHO
(6)
CHO
(1)
CHO
(2)
O
(12)
OH
(21)
CHO
(2)
CHO
(1)
OPP
(20)
+
+



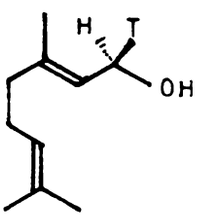
(14) (15) (13) (17) (16)

2.3 Biosynthetic relationship of citral-trans and citral-cis in lemongrass (Cymbopogon flexuosus)[14]

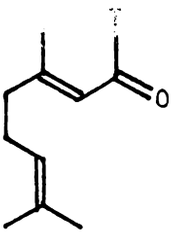
The formation of large quantities of trans and cis isomers of citral in lemongrass oil needs explanation.

The biosynthesis of citral-trans (1) and citral-cis (2) probably involve the biogenetic equivalent of geraniol (3). Four different pathways can be hypothetically visualised for the biosynthesis of (1) and (2). Firstly compounds (1) and (2) are biosynthesised by different routes from geraniol (3) and nerol (4) respectively (3 → 1 and 4 → 2) (Scheme XIII).

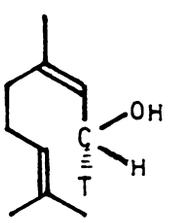
Scheme XIII



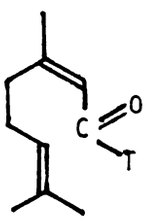
(3)



(1)



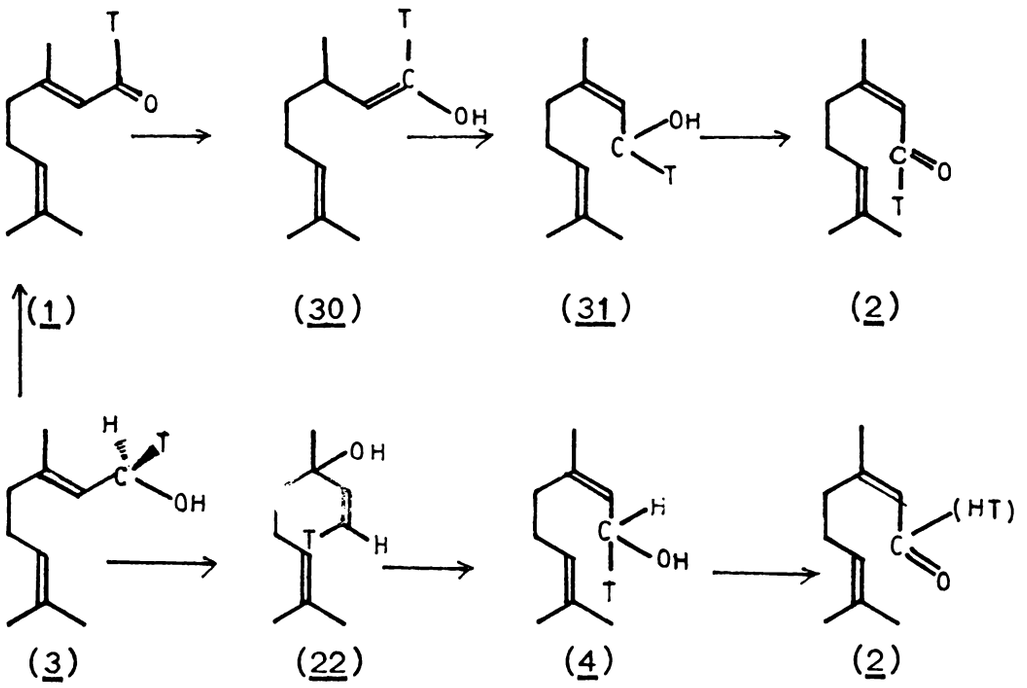
(4)



(2)

Secondly one of the aldehyde isomers gets biosynthesized from its alcohol precursor and then gets converted to the other isomer (1 → 2 or 2 → 1). The biosynthesis of (1) and (2) can also take place through two different pathways a(i) and b(i) (Scheme XIV).

Scheme XIV



In route a(i) geraniol is converted into citral trans with the loss of proton(IRH)and then metabolised into citral cis via a series of reactions (3 → 1 → 30 → 31 → 2) maintaining the same isotope ratio. Had the route been different (3 → 22 → 4 → 2) some tritium from C-I of (3) would have definitely been lost during the

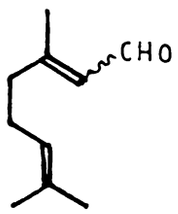
formation of the carbonyl group thus ultimately bringing down the isotope ratio, which was not observed. A reversal of the sequence of reactions was observed after feeding double labelled nerol. The isotope ratio in another experiment suggest the route 4 → 2 → 30 → 31 → 1) thus proving that all the reactions occuring leading to conversion of citral trans to citral cis are reversible. Results obtained are consistent with the suggestion that the sequence (3 → 1 → 30 → 31 → 2) and (4 → 30 → 31 → 1) exists in Cymbopogon flexuosus. When radioactive aldehyde isomers were fed to the plants they did not pass radioactivity into geraniol or nerol, thus indicating that the alcohols present in lemongrass oil (geraniol and nerol) are not produced by the reduction of citral.

C H A P T E R III

COMPONENTS OF LEMONGRASS OIL

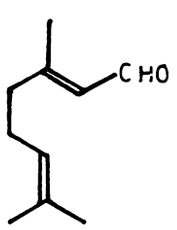
3 Components of Lemongrass Oil

3.1 Citral (3,7-dimethyl-2,6-octadiene-1-al)

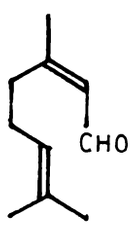


Molecular formula	$C_{10}H_{16}O$
Molecular weight	152.23

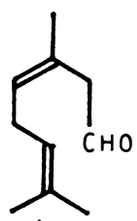
Citral is the main component of Lemongrass oil (70 to 75% of LGO). When freshly distilled, citral is an almost colourless liquid, possessing a characteristic lemon odour. Citral is a mixture of cis and trans acyclic α-β unsaturated aldehydes namely neral (1) and geranial (2). The two isomers are well characterised by NMR analysis[1,2]. In lemongrass oil geranial and neral are present generally in the ratio 5:3. When heated over 130°C, citral isomerises to isocitral[3](3).



(2)



(1)



(3)

3.1.1 Geranial

$B.P._{2.6\ mm}$ 92-93°C

d_4^{20} = 0.8888

n_D^{20} = 1.48982

Odour : Strong lemon odour

3.1.2 Neral

$B.P._{2.6\ mm}$ 91-92°C;

d_4^{20} = 0.8869

n_D^{20} = 1.48690

Odour : Sweeter than that of geranial and does not have such a pronounced lemon odour.

As a component of fragrance formulations, citral is of limited value, although its powerful lemon aroma is useful in certain compositions where a fresh note is desired. Since citral is a rather active and unstable terpenoid it may cause trouble when used in cosmetics and soap products. But in flavours citral is of paramount importance in many formulations, especially of the citrus type. The addition of citral to citrus flavours generally strengthens the flavour of natural citrus oils.

Citral has long been used for the manufacture of ionones and methyl ionones. The manufacture of

Vitamin A was previously dependent solely on natural citral, but now synthetic citral is also available. While relatively small amounts of citral as such are used in the perfume and flavour industries, it is important that citral used for such purposes be of the highest purity. For instance natural citral containing traces of impurities such as methyl heptenone is unsuitable for perfumery or flavouring. Citral prepared by the dehydrogenation of geraniol may contain appreciable quantities of citronellol which again make it unsuitable for many uses. Interestingly enough, even pure synthetic citral has its drawbacks. For example, a highly purified natural citral may consist of 99% citral. The trace substances in natural citral which make up the balance may impart the desired note in a formulation. Synthetic citral may possess a high degree of purity, but as its trace components are different, the perfumer or flavourist is encountered with a "new citral".

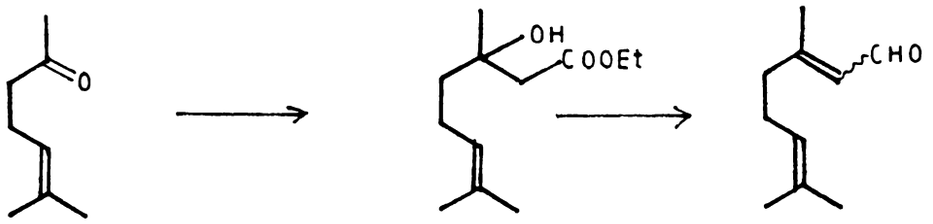
3.1.3 Isolation and identification

Bertram[4] separated citral for the first time from the oil of Backhonsia Citriodora through its bisulfite compound and in 1890. Dodge[5] separated it from Indian Lemongrass oil and gave the name citral. Citral can be identified through a number of derivatives like semicarbazone, thiosemicarbazone, citral-β-napthothocinchonic acid, citrylidenecyanacetic acid, 3-nitro- benzo hydrazone etc.[6]

3.1.4 Synthesis of citral

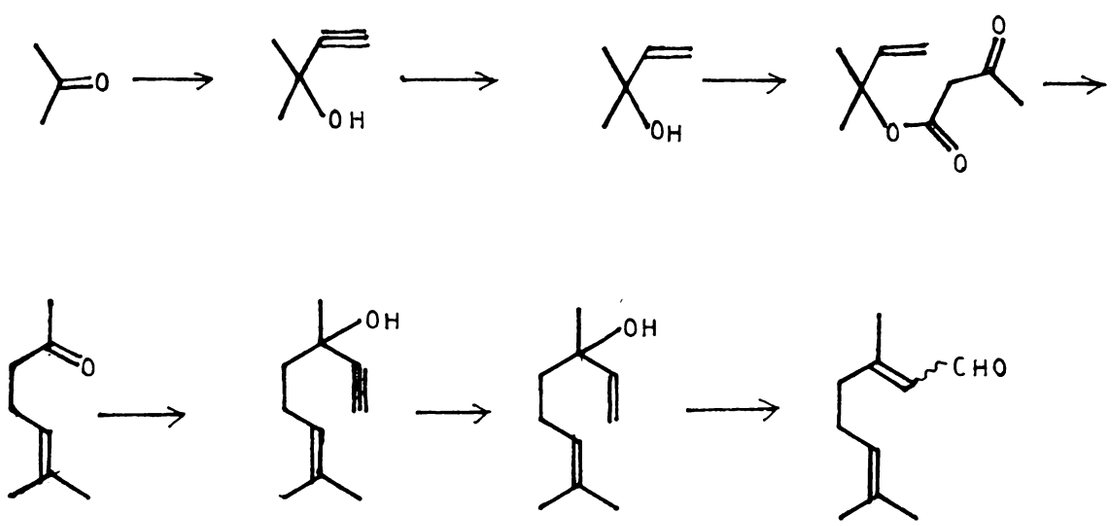
Various methods were reported in literature for the synthesis of citral[7-20]. The first synthesis of citral was conducted by Tiemann in 1898 from methyl heptenone[7]. (Scheme I).

Scheme I

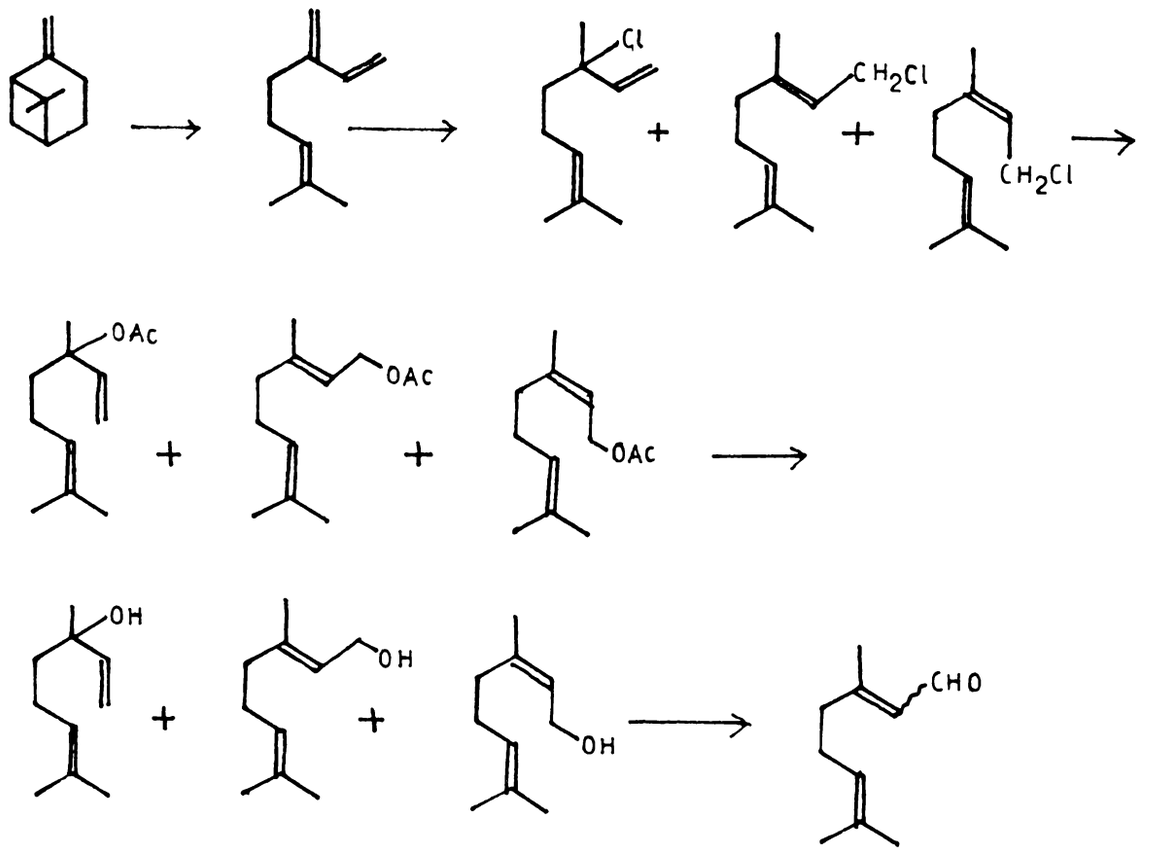


The total synthesis of citral starting from acetone[18] is given in scheme II and the industrial synthesis from β-pinene[19,20] is given in scheme III.

Scheme II



Scheme III



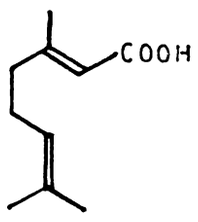
3.1.5 Important Reactions of Citral

Due to the conjugation of the ethylenic linkage, citral shows a marked exaltation in its molecular refraction.

Owing to the presence of one aldehydic and two ethylenic linkages, citral is very readily attacked by oxidising agents. Even on exposure to air, citral oxidises very easily where by it changes its colour to yellow. Under the influence of weak oxidising agents, like ammonical silver oxide geranic acid (4) is formed.

The action of strong oxidising agents like chromic acid yields geranic acid (4) methyl heptenone (5) and methyl heptenone carboxylic acid.

When citral is treated with acetic anhydride and sodium acetate, the acetate of the enol form of the aldehyde (6) is obtained, which on hydrolysis gives back isocitral (3) but not citral[21].



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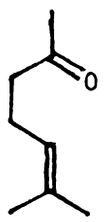
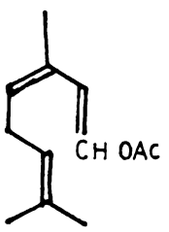
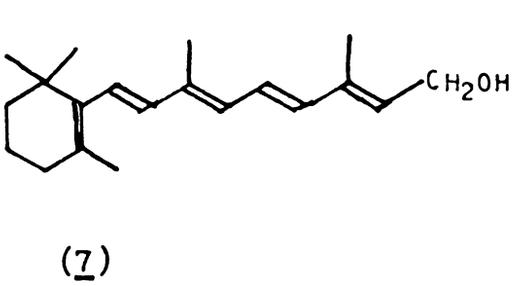


(5)

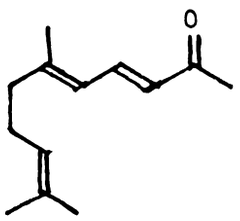


(6)

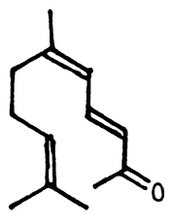
Among the most interesting properties of citral are the condensation with substances containing a reactive methylene group. These condensations have become of great importance in the synthesis of ionones and Vitamin A (7). Both isomers of citral condense with acetone in basic medium, giving pseudoionone-a (8) and Pseudoionone-b (9), which readily cyclises in acids to give a mixture of α (10), β (11) and γ-(12) ionones[22]. The odour of ionones resemble that of violets. Condensation of citral and ethyl methyl ketone results in a mixture of n- and isomethyl Pseudoionones (13 and 14), each of which may occur as four cis trans isomers[23].



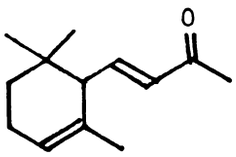
(7)



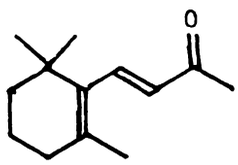
(8)



(9)



(10)



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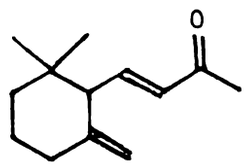

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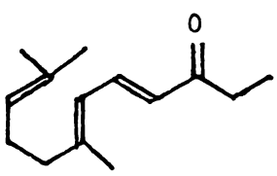

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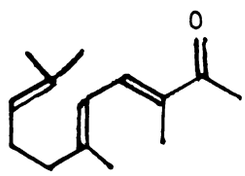
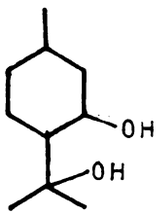


(14)

Under the influence of acids and acid media, citral readily undergoes cyclisation. Cyclisation in presence of aqueous acids gives 3,8-p-menthadiol (15). Treatment with acids converts citral to p-cymene (16). When treated with 20% H_2SO_4 citral gets cyclised to isopiperitol (17) which on further treatment with dil. H_2SO_4 isomerised to p-cymene[24] (16).



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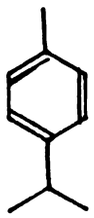

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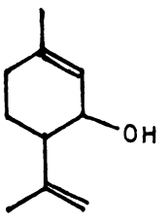
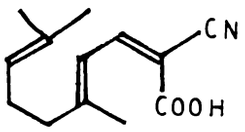


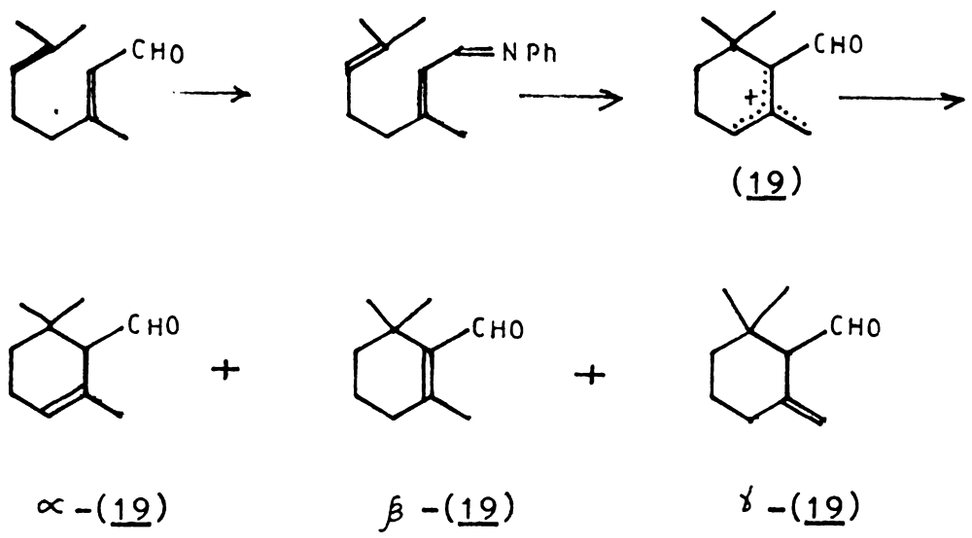
(17)



(18)

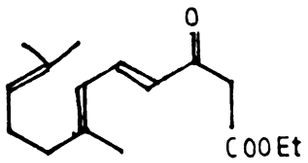
Citral cannot be converted to cyclocitral directly since it readily undergoes dehydrative cyclisation with strong acids to p-cymene. By protecting the aldehydic group either by the formation of the citryledene cyanoacetic acid (18) or by the condensation with aniline[25] it is possible to obtain a mixture of cyclo citrals (19) (Scheme IV).

Scheme IV



Citral forms acetals readily on treatment with orthoformates or ethylene glycol in presence of catalysts. High yields of acetals are formed by reacting citral with tetra-alkoxy-silanes in presence of catalysts[26]. Citral condenses with ethylaceto acetate to give citrylidene acetoacetate[27] (20) and reacts normally with bromo acetic ester to give the hydroxy acids (21) which dehydrate on heating with iodine[28].

Citral when irradiated cyclised to a mixture of a monocyclic-photocitral-a (22), and bicyclic-photo-citral-b (23)[29]. Photolysis of citral at $\geqslant 80^{o}$ gave (24)[30]. Citral when treated with alkaline H_2O_2 gives the epoxide (25) which can be converted to linalool (26)[31].



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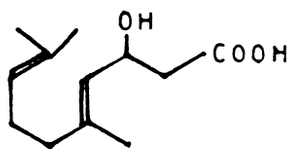

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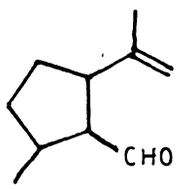
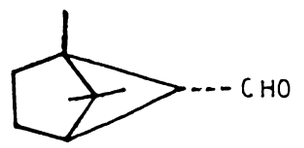
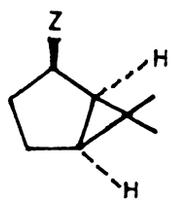


(22)

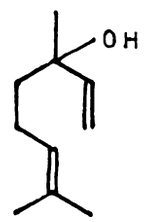


(23)



(24)

(Z = ∝-Me,
β-CHO,
∝-CHO,
β-Me)



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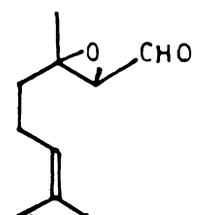
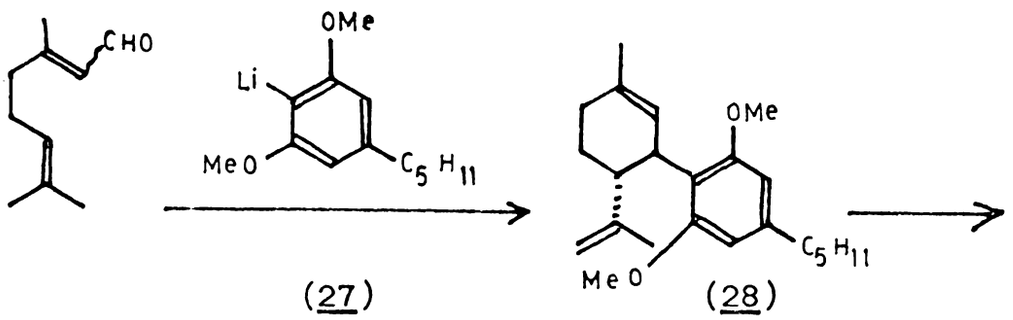


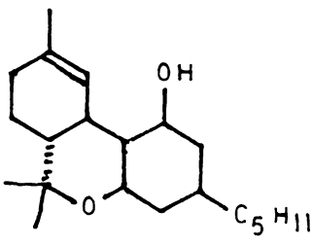
(25)

Citral when treated with sodium salt of Olivetol dimethyl ether (27) followed by reaction with p-TsCl afforded Cannabidiol-dimethyl ether (28) in 7% yield, which was converted into Δ^{1}-Tetrahydro Cannabinol (29)[32,33] (Scheme V).

Scheme V



(27) (28)

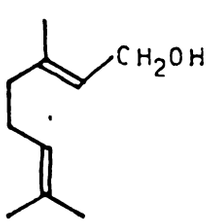


(29)

A number of studies have been made on the reduction of citral[34,39], yielding different products like geraniol (30),nerol (31), citronellal (32), citronellol (33), tetrahydrogeraniol (34), Pinacone(35) and 3,7 dimethyl octane (36).

The oxime of citral (37) on dehydration gives geranonitrile (38)[40].

Diethyl acetal of citral on refluxing with NBS in CCl_4 gave p-cymene (16) in 30% yield[41]. The ketal of citral on SeO_2 oxidation also gave p-cymene (16)[42].



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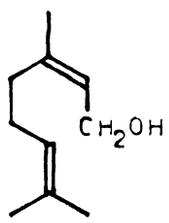
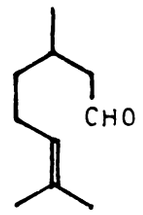
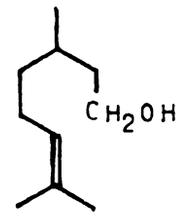


(31)



(32)



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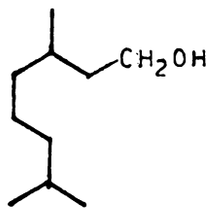
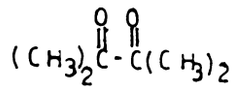
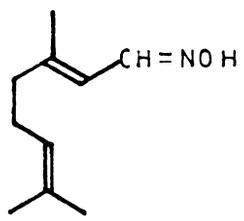


(34)

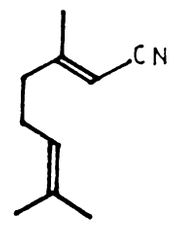


(35)

(36)



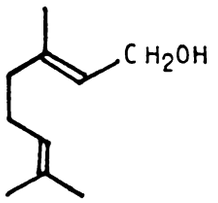
(37)



(38)

Another important property of citral is its ability to form adducts with sodium sulfite and sodium bisulfite. These reactions and their side reactions will be discussed in Chapter IV.

3.2 Geraniol (2 trans-3,7-dimethyl-2,6-Octadien-1-ol)



Molecular formula	:	$C_{10}H_{18}O$
Molecular weight	:	154.24
B.P.$_{12\ mm}$	:	114-115^{o}
Refractive Index at 20^{o}C	:	1.4690 to 1.4780.
Specific gravity	:	0.870 to 0.885
Flash point	:	103^{o}

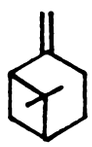
Pure geraniol is a colourless, very pleasant smelling liquid, having a sweet, rose odour. When exposed to air, geraniol, discolours and its odour gradually deteriorates due to absorption of oxygen. It is an unsaturated primary terpene alcohol with two ethylenic linkages and is isomeric with nerol and linalool. It is one of the most widely used perufumery chemicals in soaps, detergents and cosmetics. Geraniol is valuable wherever fresh rose notes are desired in fragrance formulations.

In the past all methods of preparation of geraniol depended on its separation from natural sources. One of the earliest methods involved the formation of $CaCl_2$ addition compound.[43] Geraniol is identified by its derivatives like diphenyl methane, β-naphthyl urethane,

phenyl urethane, 3-nitrophthalate.

Nowadays it is prepared from myrcene (39) which is turn can be obtained from β-pinene (40). In recent years 96% pure synthetic geraniol was prepared by isomerisation of linalool using orthovanadates as catalysts[44].

(39)

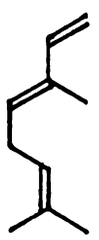


(40)

Because of the two ethylenic linkages geraniol is a highly reactive substance. With sodium bisulfite it forms a stable compound $C_{10}H_{18}O$. 2 $NaHSO_3$ from which geraniol cannot be regenerated by alkali. Action of mineral acids and dehydrating agents on geraniol is very diverse and the products depend greatly on the experimental conditions. The action of acid reagents may bring about cyclisation and formation of cyclogeraniol, however geraniol is more stable towards acids than linalool. In the cold, alkalies do not act on geraniol. Oxidation of geraniol with chromic acid gives mainly citral along with a little of methyl heptenone (5). Oxidation with very dilute $KNnO_4$ solution yields first a polyhydric

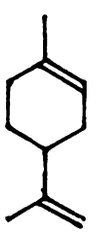
alcohol and finally various products of complete degradation.

Dehydration of geraniol with RN=C=NR'(R=cyclohexyl, R'=p-ClC_6H_4) gave mainly the acyclic hydrocarbons myrcene (39), transocimene (41), and cisocimene (42), whereas nerol gave mainly dipentene (43) and terpinolene (44). Persumably due to the relatively slow cis-trans isomerisation of the cations of the starting materials[45]. Oppenauer oxidation of geraniol gave pseudoionones (8 and 9)[46].

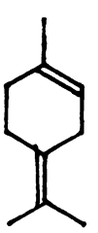


(41)

(42)

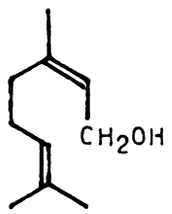


(43)



(44)

3.3 Nerol (2-cis-3,7-dimethyl-2,6-octadien-8-ol)



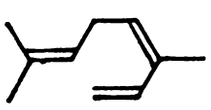
Molecular formula	:	$C_{10}H_{18}O$
Molecular weight	:	154.24
B.P.$_{760\ mm}$	:	224-227°C

Nerol possess a pronounced rose like odour but more refreshing than that of geraniol.

Nerol can be isolated from the terpene alcohol fraction of volatile oils, after removing the geraniol as its $CaCl_2$ addition product. It is then further purified by converting nerol to its crystalline diphenyl urethane. Hydrolysis with alcoholic potassium hydroxide solution yields pure nerol.

Reactions of nerol are almost similar to gerniol. Contrary to gerniol it does not form a crystalline compound with $CaCl_2$.

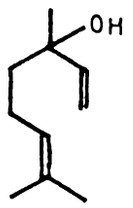
A 2:1 E/Z mixture of (45) on treatment with active Mg and B $(CoBu)_3$ in THF followed by alkaline H_2O_2 gave 30% of a 1:1 mixture of nerol (31) and linalool (26)[47].



(45)

Nerol is used as a valuable constituent in synthetic rose and orange blossom perfumes. However, the high price of nerol restricts its use in cosmetics and soaps.

3.4 Linalool (3,7 dimethyl-1,6-octadiene-3-ol)



Molecular formula	:	$C_{10}H_{18}O$
Molecular weight	:	154.25
B.P.$_{760\ mm}$	:	198^{o}
d_4^{20}	:	0.8700
n_D^{20}	:	1.4616

(±) Linalool is a colourless liquid with a flowery fresh odour, quite different from its isomers geraniol and nerol. Having a lower boiling point than its isomers it serves as a natural and desirable top note in perfumes.

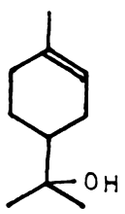
Linalool can be prepared from methyl heptenone and also from ∝-pinene[48] and β-pinene[50].

Chromic acid oxidation of linalool yielded citral, acetone and methyl heptenone[51]. It can be readily reduced to both the saturated alcohol and the corresponding hydrocarbon. It on prolonged heating with acetic anhydride, isomerises to geraniol, the

isomerisation is reversible in the presence of water at high temperature and in the absence of catalysts[52].

It undergoes cyclisation very easily. Both dilute sulfuric acid and strong formic acid converted linalool to geraniol (30), nerol (31) and α-terpin-hydrate (46).

Treatment of linalool with 30% H_2SO_4 at elevated temperatures gave myrcene (39),dipentene (43), terpinolene (44), p-cymene (16) α-terpineol (47), 1:4 cineole (48) and 1:8 Cineole (49)[54].



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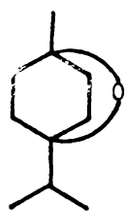

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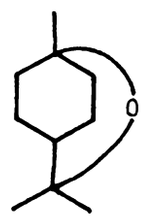
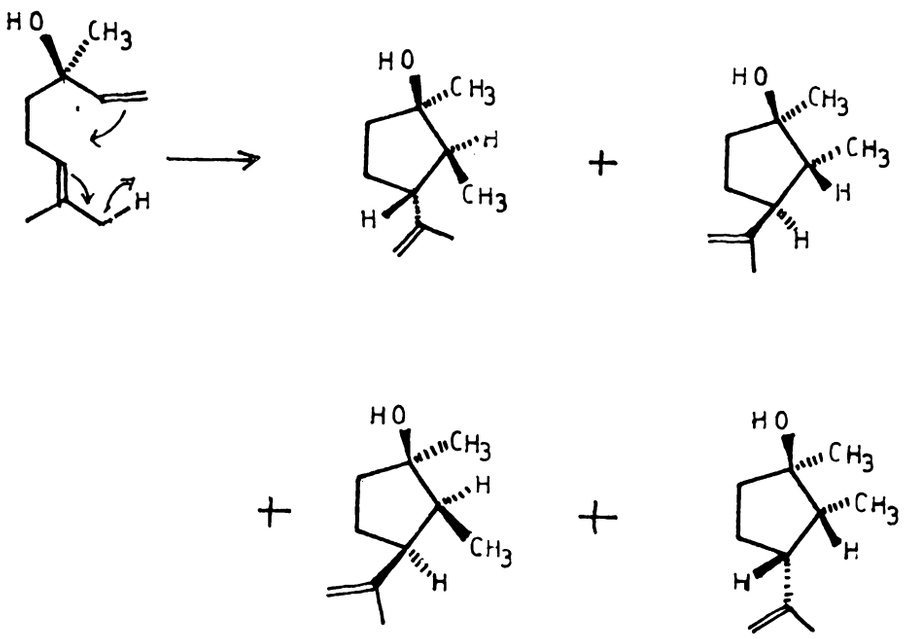


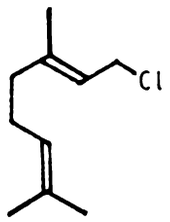
(49)

Plinols can be obtained from linalool by dehydration[55] (Scheme VI).

Scheme VI



Linalool can be converted to a mixture of geranyl chloride (50) and linalyl chloride (51) containing 25-40% of (51)[56]. Hydrogenation of dehydrolinalool to linalool over 0.5% Pd-Al_2O_3 in C_2-C_5 primary alcohols gave 99 to 100% yields with a selectivity of 100%. By increasing the catalyst content to 50%, 72-83% linalool with a selectivity of 83.5 - 91.2% is obtained[57].



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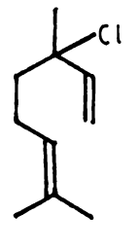
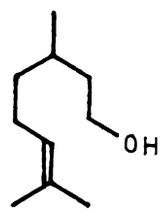


(51)

3.5 Citronellol (3,7-dimethyl-6-octen-1-ol)



Molecular formula : $C_{10}H_{20}O$

Molecular weight : 156.27

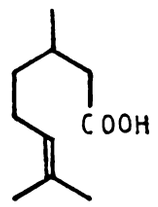
B.P.$_{760\ mm}$: 224.5°

Citronellol is a colourless liquid with a sweet rose like odour.

Various methods were reported for the preparation of citronellol[58-63].

Citronellol can be oxidised to the corresponding aldehyde (32) and acid (52) whereas more vigorous reagents cause rupture at the ethylenic linkage. Citronellol can be subjected to hydrogenation under

varying conditions to give dihydro citronellol (53) and 2,6-dimethyl octane (54). Citronellol readily forms its esters.



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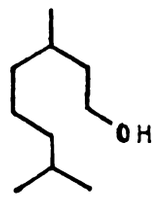
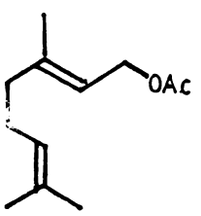


(53)

(54)

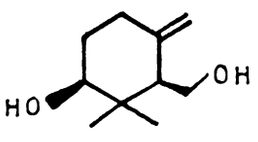
3.6 Geranyl acetate



Molecular formula	:	$C_{12}H_{20}O_2$
Molecular weight	:	196.29
B.P.$_{15\ mm}$	:	98^{o}

Geranyl acetate is a colourless liquid with a pleasant fruity rose note reminiscent of Pear and slightly of lavender. It can be prepared by the acetylation of geraniol. Geranyl acetate on treatment with benzoyl peroxide, cupric benzoate and cupric chloride in acetonitrile resulted in the cyclisation

and the resulting mixture was hydrolysed to the corresponding diols, from which the cis diol (55) was separated by chromatography. (55) on treatment with one equivalent of p-toluene sulfonyl chloride in pyridine at room temperature gave racemic Karahana ether (56)[64].



(55)

(56)

3.7 Myrcene (7-Methyl-3-methylene-1,6-octadiene)

Molecular formula : $C_{10}H_{16}$

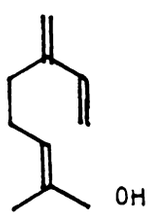
Molecular weight : 136.23

B.P.$_{760\ mm}$: 167^{o}

Various methods are reported in the literature for the synthesis of myrcene[65,66,67]. On an industrial

scale it is prepared by the pyrolysis of β-pinene (40)[68,69].

On standing myrcene undergoes polymerisation both to a dimer di myrcene ($C_{20}H_{32}$) and also to a polymer-polymyrcene $(C_{10}H_{16})_x$. When treated with $KMnO_4$, myrcene is oxidised to succinic acid (57)[70] and with seleniumdioxide[71] to myrcenol (58), myrcenal (59) and myrcenic acid (60). In presence of catalyst containing 1% K_2O_3, 5% Cr_2O_3, 5% of a mixture of oxides containing 2.7% CeO_2, 1.3% La_2O_3, 0.7% Na_2O_3 and 0.3% Pr_2O_3 and 89% Al_2O_3 and H_2S at 450°C yielded p-cymene (16) in 76.5% yield[72]. Acid catalysed addition reaction of myrcene with NBS and ROH (R=H, CH_3, C_2H_5, Me_2CH-, cyclohexyl) occured exclusively at the isolated double bond rather than at the conjugated double bond to give (61)[73].



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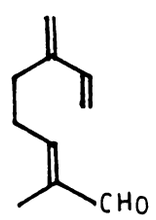

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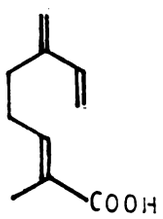

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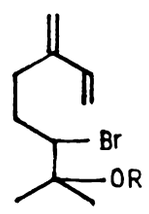
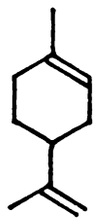


(61)

3.8 Limonene (1,8-p-Menthadiene:1-methyl-4-Isopropenyl-1-cyclohexane)

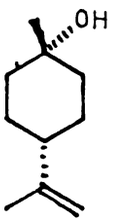


Molecular formula : $C_{10}H_{16}$

Molecular weight : 136.23

B.P.$_{760\ mm}$: 177.6 - 178°

Limonene is a colourless oil possessing a pleasant orange odour. Presence of air and light oxidises it readily. It occurs in the d and l forms. When optically active limonenes are heated or treated with acids racemic form dipentene is formed. Both dipentene and limonene are present in lemongrass oil. (+) R Limonene can be converted into - IS, 4 R -p-mentha-2,8-diene-1 -ol,- (62)[74]. Treatment of diastereomeric epoxides (63) obtained as a mixture by the epoxidation of (-) R Limonene with Na eph gave a mixture of (64) which was oxidised directly with H_2O_2 to the corresponding selenoxides (64) and (65). (65) was converted to (-) IS, 4R p-mentha-2,8-diene-1-ol (+62).



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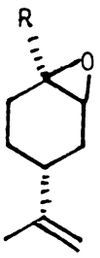

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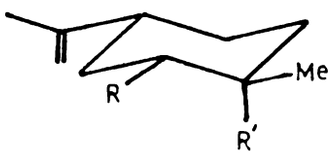

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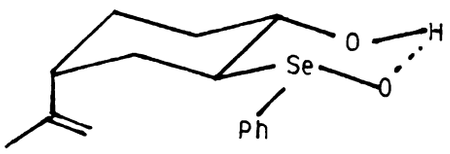

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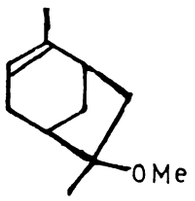

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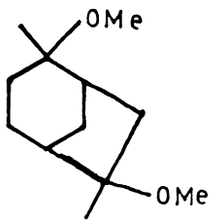
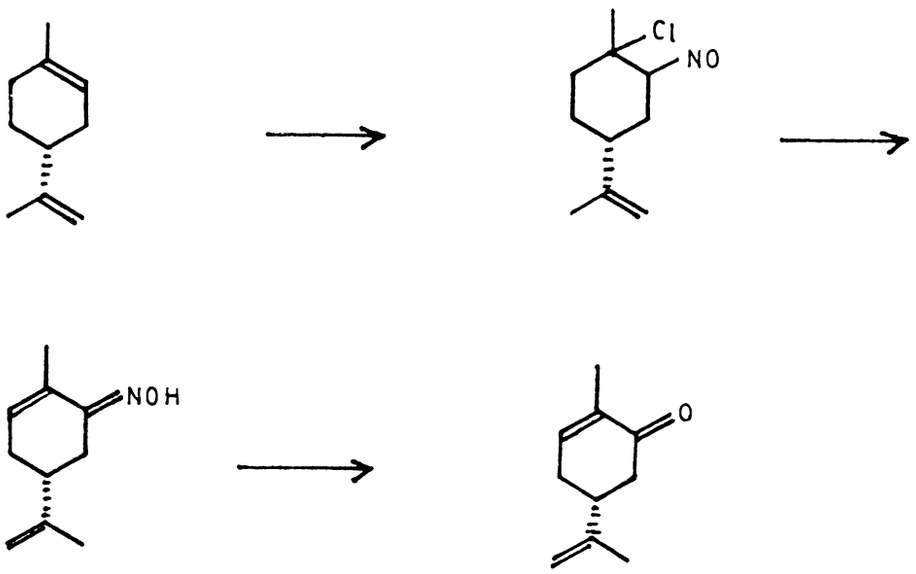


(67)

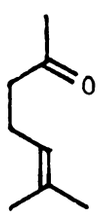
Limonene when treated with thallium nitrate in MeOH gave (66) and (67)[75].

Limonene can be converted to (-) carvone (68)[76] (Scheme VII).

Scheme VII



3.9 Methyl heptenone (6-Methyl-5-hepten-2-one)



Molecular formula : $C_8H_{14}O$

Molecular weight : 126.19

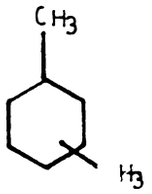
B.P.$_{760\ mm}$: 173-174°

It is a colourless, mobile liquid possessing a peculiar characteristic odour, which is not impressive. It is an

important intermediate in the synthesis of terpenoids. In nature methyl heptenone occurs as a decomposition product of terpenoids.

On oxidation methyl heptenone yields acetone and can be prepared from citral by the retroaldol reaction. Methyl heptenone is sensitive to acids and can undergo cyclisations to hydrogenated xylenes (69) and tetrahydro pyrans (70).

Methyl heptenone was converted to Levandulol (71)[77].



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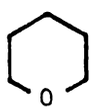
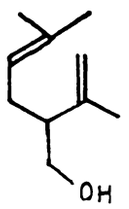
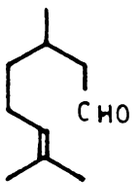


(70)



(71)

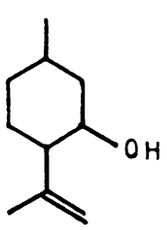
3.10 Citronellal (3,7-dimethyl-6-octen-1-ol)



Molecular formula	:	$C_{10}H_{18}O$
Molecular weight	:	154.25
B.P.$_{760\ mm}$	:	207-208^{o}

Pure citronellal is a colourless liquid with a refreshing odour. It exist in d, l and dl forms. (±) citronellal can be prepared from geraniol/nerol by the vapour phase rearrangement in the presence of barium containing copper-chromium oxide catalyst[78]. It can also be prepared by the dehydrogenation of citronellol under reduced pressure with a copper chromite catalyst[79] and also from citral by the partial hydrogenation with a lower aliphatic alcohol as the solvent, in the presence of a palladium or a chromium activated Raney Nickel catalyst[80]. Isopulegol (72) on pyrolysis will also give citronellal[81].

After protection of the aldehyde group, addition of water to the double bond in presence of mineral acids or ion exchange resins results in the formation of hydroxycitronellal (73). Citronellol can also be converted to (-) Menthol (74) through isopulegol. By the action of acetic anhydride, isopulegol-acetate (75) is formed. Under the influence of alkalies, citronellal resinifies rapidly. Enzymatic reduction of citronellal to citronellol is also reported[82].



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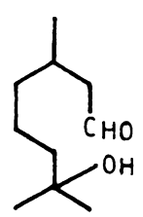

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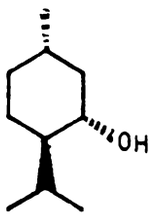

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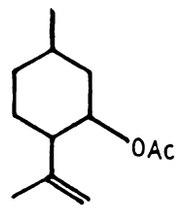
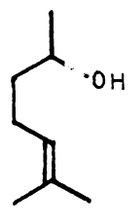


(75)

3.11 Methyl heptenol(6-methyl-5-hepten-2-ol)[83]



Molecular formula : $C_8H_{16}O$

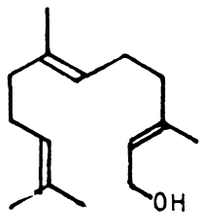
Molecular weight : 128.21

B.P.$_{760\ mm}$: 178-180°

Synthetic dl-methyl heptenol was prepared from methyl heptenone, geranic acid nitrile and geraniol[84].

It was isolated from East Indian Lemongrass oil by using the phthalic acid ester method, on the fraction with B.P. 65 - 70° of the oil.

3.12 Farnesol (3,7,11-trimethyl-2,6,10-dodecatrien-1-ol)



Molecular formula	:	$C_{15}H_{26}O$
Molecular weight	:	222
$B.P._{3\ mm}$	:	120^{o}

Of the four possible isomers (trans trans, cis cis, trans cis and cis trans), the trans trans isomer is the most common in nature. It is particularly suited for use in flower compositions and is valued for its fixative properties. Its presence is reported in some lemongrass oil like OD[19].

3.13 n-Decyl aldehyde (Decanal)

$$CH_3(CH_2)_8CHO$$

Molecular formula	:	$C_{10}H_{20}O$
Molecular weight	:	156.26
$B.P._{755\ mm}$	:	$207\text{-}209^{o}$ (with slight decomposition)

n-Decyl aldehyde was isolated through its sodium bisulfite adduct and identified by preparing derivatives

like thio semicarbazone, 2,4-dinitrophenyl hydrazone and p-iodo benzoyl hydrazone.

Owing to the powerful odour n-decyl aldehyde play a very important role in the odour and flavour of the oil, even when present in traces.

S E C T I O N A

ANALYSIS OF LEMONGRASS OIL

C H A P T E R IV

INTRODUCTION

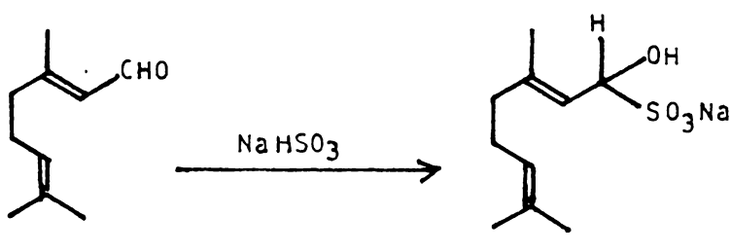
4. Analysis of Lemongrass (Cymbopogon flexuosus) Oil

4.1 Introduction

The quality of lemongrass oil is determined by its citral content. Various methods were reported in literature for the estimation of citral in lemongrass oil[1,2,3] and also for the separation of citral from lemongrass oil[4,5,6,7]. The common methods for the estimation and separation of citral are discussed below:

4.1.1 Bisulphite method [1,4]

The bisulphite method is based on adduct formation process as shown below:

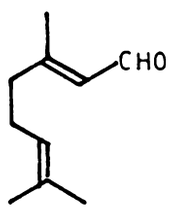


Upon shaking a measured quantity of the oil with a hot aqueous solution of sodium bisulfite, an adduct is formed, which dissolves on heating the solution. The noncitral portion of the oil separates as an oily layer which can be measured conveniently in the neck of a Cassia flask and thereby determine the citral content of the oil. In the case of citral (1) due to the presence of two ethylenic linkages, and an

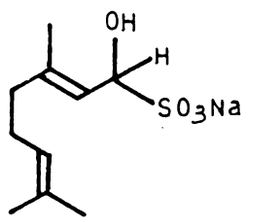
aldehyde group, the adduct formation is complex. A normal addition compound (2) is formed when one molecule of sodium bisulfite combines with the carbonyl group of citral, from which citral can be regenerated by treatment with alkalies such as sodium carbonate. A labile disulfonate (3) is formed in which the sulfonate radical is apparently attached to carbonyl group and also to the ethylenic linkage conjugated to the carbonyl group. The disulfonate adduct formed is water soluble and citral can be regenerated from it by treatment with sodium or potassium hydroxide. But there will be a loss of 10 to 15% in the recovery. With sodium bisulfite for several hours in acid medium citral give a disulfonate(4) which is stable and from which citral cannot be regenerated. Since it combines with phenyl hydrazine, it contains a free aldehyde group. The sulfonate radical evidently shifts on altering the acidity of the solution. Thus the labile disulfonate gets converted to the stable derivative by treatment with acids.

According to Dodge[6], it is possible to obtain besides the normal addition prdduct, a water soluble monosulfate where the sulfonate radical is attached to the double bond conjugated to the carbonyl group. The carbonyl compound can be regenerated from this product. However in the case of citral, if the sulfonate radical is attached to position 7(5), the compound is stable and

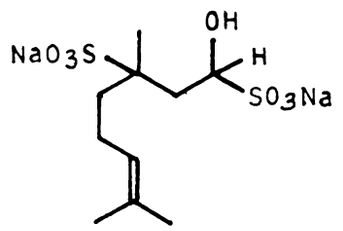
from it citral cannot be regenerated. A mixture of these three types may also be formed simultaneously and theoretically it is possible to get seven sulfonates. Thus any mono or poly sulfonate which has a sulfonic radical in position 7 of the citral molecule is a stable compound from which citral cannot be regenerated, thus having sulfonate groups attached to positions 1,3 or 7. Citral can be regenerated if the sulfonate groupings are at 1,3 or 1 and 3, but cannot be regenerated if the sulfonate groupings are at 1,7 and 1,7 and 3 or 7 and 3 and 7 and 1.



(1)



(2)



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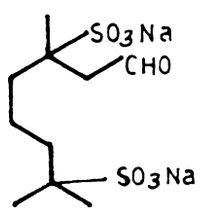

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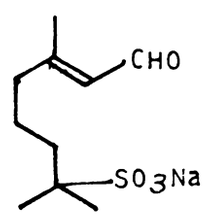


(5)

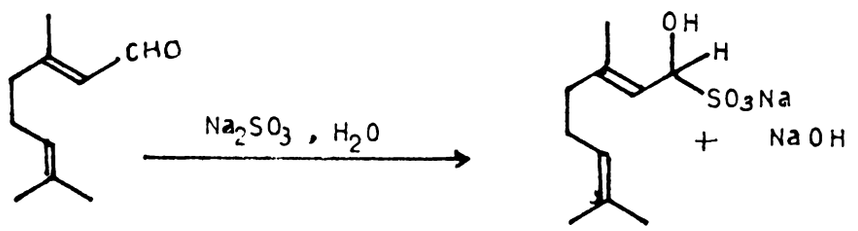
Commonly used method of estimation of citral especially in trade and commerce is the bisulfite adducting method. But this method has many disadvantages as an estimation method for citral in lemongrass oil. Since lemongrass oil contains aldehydes other than citral like citronellal, n-decyl aldehyde etc. and also methyl ketones like methyl heptenone and all aldehydes and methyl ketones will get adducted with sodium bisulfite, the value obtained will be much higher than the actual citral content.

As a method of separation also this method has many defects. All the aldehydes and methyl ketones present in lemongrass oil will get adducted and on regeneration all of these components will be regenerated thereby reducing the purity of citral obtained. In this process, the oil is shaken with saturated sodium bisulfite solution - and the resulting crystalline solid is separated and purified by washing with alcohol or ether. Citral is regenerated by decomposition of the adduct with sodium carbonate, sodium hydroxide or hydrochloric acid. Eventhough the normal adduct can be easily decomposed, quantitative regeneration of citral is difficult. Usually a loss of 10-15% is observed. The loss is reported to be due to the formation of a cyclic bisulfite compound in presence of alkali from which recovery of citral is found to be difficult[8]. Acid

initiated decomposition of the adduct usually leads to cyclisation and polymerisation of the product which is undesirable. Because of these reasons the yield as well as the purity of the citral separated will be poor.

4.1.2 Neutral sulfite method[2]

This is also an adduct formation reaction as shown below:

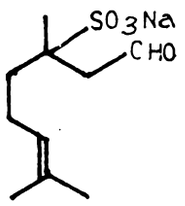


In this method, sodium hydroxide liberated has to be neutralised periodically with acid to permit the reaction to go to completion. Neither must the solution be permitted to turn acidic, as this would result in the formation of the stable dihydrosulfonic compound(4), from which citral cannot be regenerated. For this purpose, Tiemann[9] suggested the following modification.

Shake a solution of 350 gm of sodium sulfite in 1 litre of water with 100 gm. of pure citral, after adding a few drops of phenolphthalein. Reduce the strongly alkaline reaction to be produced, from time to time by gradually adding standardised sulfuric acid of about 20% strength. Keep the solution always slightly alkaline.

This method also has all the disadvantages of the bisulfite method as a method of estimation and also of separation. However it offers certain advantages over bisulfite method. By using the indicator (phenolphthalein) the exact end point of the reaction can be determined[2].

Erman and Coworkers[10] found that when citral was treated with Na_2SO_3 in aqueous base at pH 11.2, (6) was obtained in 77% yield, which was cyclised to give 81%(7). At pH 3 - 8.8, 87% (3) was obtained. Additional products obtained were (2), (4) and (8).



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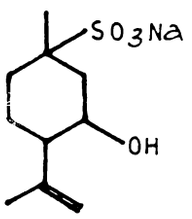

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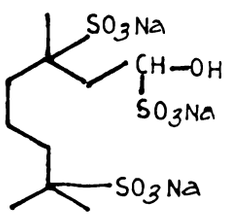

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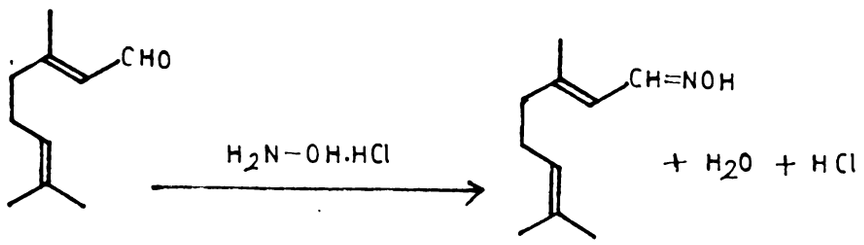
4.1.3 Hydroxylamine Method[11,12]

This method is also used for the estimation of citral in lemongrass oil. This method makes use of both hydroxylamine and hydroxylamine hydrochloride and is based on the equation,



After the reaction of this with the carbonyl group the mixture is titrated with standard alkali.

The hydroxyl amine method also has some defects. All the carbonyl groups present in lemongrass oil will react with hydroxyl amine and the value obtained will be much higher. However this method offers some advantages over the adduct formation process.

1. Relatively small amounts of the oil are required for an estimation.
2. The reaction of hydroxylamine with aldehyde is rapid, thereby shortening the time required for the estimation.
3. This methods proves exceptionally applicable to oils which contain large amounts of aldehydes.
4. The solution used for the standard procedure are stable and can be kept for longer periods.

The titration can also be done potentiometrically. For that known amount of titrant are added to the solution to be titrated. After each addition, the pH is determined and end point of titration found out. The end point is the increase in volume with highest pH difference.

4.1.4 Colorimetric Method[3]

The citral content of lemongrass oil has also been estimated by the coloring agent - that of Ehrlich Miller. This coloring agent has been found to give better results and development of colour takes place rapidly and remain quite stable for a long time. The colouring agent has been prepared according to Ehrlich Miller and consist of the solutions.

1. 5% p-dimethylaminobenzaldehyde solution in acetic acid.
2. 10% phosphoric acid solution in acetic acid.

One ml each of the above solutions are added to different amounts of citral in acetic acid, whereby a marked colour change from blue to pink can be observed. The percentage absorbance and extinction of the coloured citral is then measured using colourimeter and calibration graphs are ploted. The amount of citral in solutions can be compared with that of known strength and thus the percentage of citral can be determined.

Here also we need solutions of citral with known strength.

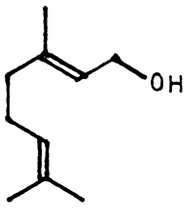
Since all these methods discussed have defects as a method of estimation of citral, it was thought to develop a new method of estimation of citral in lemongrass oil, having no defects. With this aim in view a new method for the estimation of the correct percentage of citral in lemongrass oil by physical separation of citral quantitatively in pure form, was developed by column chromatographic technique[13].

Preliminary experiments were done using neutral alumina (grade I) as the adsorbant and hexane as the eluent. Eventhough it was possible to separate all the hydrocarbons using this system, it was found that on prolonged column chromatography, the column developed heat and the chromatography could not be done satisfactorily. The development of heat can be attributed to the polymerisation of the highly reactive α-β unsaturated aldehydes namely geranial and neral on the alumina column. Hence further chromatographic studies were undertaken using the milder adsorbant silicagel. Preliminary studies gave promising results. After repeated column chromatographic studies it was found possible to isolate citral quantitatively in pure form (99+% - purity checked by GLC) and thus determining the correct percentage of citral in lemongrass oil. This

happens to be a new method of estimation of citral.

Different varieties of lemongrass oil obtained from different regions of India (RRL - Jammu, RRL - Bhubaneswar, RRL - Jorhat, Odakkali, Bhuttan and Cochin) were analysed by the newly developed column chromatographic technique and the correct percentage of citral present in these oils were determined.

For the separation of citral from lemongrass oil both chemical and physical methods are used at present. The chemical methods used were discussed earlier in the bisulfite and neutral sulfite adducting methods. On an industrial scale it is commonly separated from lemongrass oil till now by vacuum fractionation of the oil. In vacuum fractionation an enrichment of citral happens and citral of only 95% purity is generally obtained. Moreover removal of components like geraniol(9) nerol (10) etc., which have boiling points differing only by few ^{o}C from that of citral is found to be difficult even when high efficiency fractionating columns are used. Being a mixture of α-β unsaturated aldehydes, citral is heat labile and excessive heat treatment is likely to lead to rearrangements, polymerisation and eventual destruction of the material. Hence in this method there is invariably a loss in the yield.



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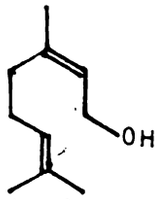

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Hence in both the vacuum fractionation and in the adducting methods, used at present for the isolation of citral from the oil, the purity as well as the yield of citral is unsatisfactory. Since estimation of the correct percentage of citral in lemongrass oil by the physical separation of pure citral in quantitative yields was achieved, column chromatographic method for the separation of citral from lemongrass oil was tried. With a view to check the possibility of commercialising the isolation of pure citral in quantitative yields from lemongrass oil, slight modifications were made on the estimation method using column chromatography. Changes were made on the column parameters like the ratio of adsorbant to silicagel, eluents and rate of elution. It was found possible to isolate pure citral, (99+% purity checked by GLC) in near quantitative yields by this method[14]. This process being a physical process not involving the use of any chemicals, the possibility of rearrangements of citral during separation is minimal. This process also

excludes excess of heat treatment which is undesirable in the case of thermally labile molecules like citral. The adsorbant used can be regenerated and reused after proper cleaning and activation and also the eluents used can be recovered and recycled. Because of these advantages this method is far superior to other existing physical (distillation) and chemical methods, available, for the separation of citral of high purity and that too in near quantitative yields.

Total analysis of lemongrass oil was also tried using column chromatographic method. In a single column it was possible to separate lemongrass oil into four fractions - namely mixture of hydrocarbons, gernayl acetate together with carbonyl components other than citral, citral and mixture of alcohols. The third fraction obtained was pure citral. The other three fractions were repeatedly chromatographed so as to separate individual components.

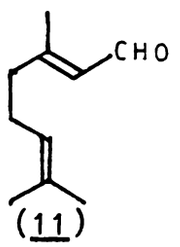
The first fraction was found to be a mixture of 3 hydrocarbons namely myrcene, limonene and dipentene. It was found difficult to separate these three components. On a long column using silicagel as adsorbant and hexane as eluent, myrcene was separated from this mixture.

8% of the lemongrass oil (commercial sample from Cochin) was found to be a mixture of 4 components namely n-decyl aldehyde, citronellal, geranyl acetate and

methyl heptenone. From this mixture pure geranyl acetate and pure methyl heptenone were separated.

12.33% of the lemongrass oil analysed was found to be alcohols - linalool, geraniol, nerol, citronellol and methyl heptenol. By column chromatography linalool was obtained in pure form. Using column chromatography the non-citral portion of lemongrass oil and also the ionone tops were analysed. They are used as cheap perfumes in soaps. On chromatographic analysis, it was found that 50-60% of these fractions are valuable alcohols. If these alcohols can be separated these fractions will command more attractive price. With this target in mind we separated the alcohols present in the non-citral portion as well as that in the ionone tops.

Citral the main component of lemongrass oil is known to be amixture of geranial (11) and neral (12) in the ratio of 5:3. Complete separation of these two isomers has not yet been reported eventhough isolation



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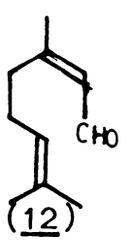


(12)

of one isomer by chemical method is claimed[4,15,16,17,18,19]. It is reported that geranial can be obtained free from neral, during regeneration from the bisulfite adduct by

taking advantage of the fact that crystalline sodium bisulfite adduct of gernial is sparingly soluble while the corresponding adduct of neral is readily soluble in water[4,9]. By this method the yield of geranial obtained will be less since its adduct is sparingly soluble in water and also neral cannot be regenerated. It is also reported that neral can be isolated from citral by shaking for a short time with alkaline cyanoacetic acid solution. Geranial is said to react with this acid much more readily than neral[16]. This separation is also not likely to be complete. Separation of these two isomers were also reported through their semicarbazones[17]. Semicarbazones of geranial and neral differ in melting points (semicarbazone of geranial M.P. 164°C and semicarbazone of neral M.P. 171°C).

By repeated recrystallisation it was possible to separate the two semicarbazones. Partial separation of citral isomers by vacuum spinning band distillation in small quantities was also reported recently[20]. By this method,of the six fractions collected, the sixth fraction contains above 95% geranial.

With a view to separate the cis-trans isomers in pure form andwithout loss of either of the isomers, column chromatographic studies were undertaken. Preliminary results indicated the possibility of separation of the two isomers. The initial experiments showed a reveral of the ratio of the two isomers in GLC. Finally

separation of citral into pure neral, mixture of neral and geranial and pure geranial was achieved[21]. Complete separation of these two isomers in quantitative yields can be considered as the ultimate achievement in the field of lemongrass oil technology. This work is of great relevance commercially since neral is highly priced and also nerol obtained by the reduction of neral is an expensive perfumery chemical. Source for pure neral and nerol in India are rare.

Column chromatographic separation of the cis-trans isomers were also checked in the alcohol level. But itwas found to be more difficult than in the case of aldehydes. For this citral was reduced using aluminium isopropoxide in anhydrous isopropanol. The product obtained on analysis showed the presence of 20% hydrocarbons and the rest being alcohols (geraniol/nerol). Hydrocarbons were formed as the side product of Meerwein Pondroff Verley reduction. Finally the product was separated into hydrocarbons, pure nerol, mixture of nerol and geraniol and pure geraniol.

C H A P T E R V

EXPERIMENTAL

5. Experimental

All gas chromatographic analysis were done on Hewlett packard 5730A Gas Chromatograph with Hewlett Packard 3390 A Reporting Integrator. (Column - 6 ft, 10% SE-30 on chromosorb, oven temperature 150°C isothermal, Injection port temperature 200°C, Detector temperature : 250°C, N_2 (40 ml/minute) FID detector).

Complete removal of solvents were accomplished using Buchi EL-130 Rotavapor. Solvents used were dried before use.

For TLC Silicagel G (Merck India) was used. Eluents used were dried before use.

5.1 Column Packing and Chromatographic conditions

Silicagel was packed by the slurry packing method using hexane as suspending medium. Suction was applied at the bottom to get uniform packing. The column elution rate was maintained throughout the process using a constant pressure head.

5.2 Method

The separation was done in a single stretch at room temperature (28°C). Small fractions were collected and checked by TLC. Fractions having same Rf values in TLC and same retention time in GLC were combined and

solvent removed at temperatures not exceeding 40°C under reduced pressure.

5.3 A new method of estimation of citral in Lemongrass oil by physical separation of citral

5.3.1 Procedure (Sample, RRL-16 - Jammu)

Silicagel (BDH : Mesh size 60-120) was used as such after activation at a temperature of 100°C for 1 hour. Solvent grade hexane and ether were used as eluents after drying. Glass columns (75 x 2.5 cm I D) were used for the estimation. Ratio of substance to adsorbant used was 1:35.

Fractions were collected in small portions and analysed by TLC and GLC. Fractions with same Rf value in TLC and same retention time in GLC were combined and solvent removed and weighed.

Column chromatographic details are given in Table I.

20 strains of lemongrass oil collected from different regions of India were analysed using the above procedure. Results are given in Table II. For comparison, citral content was also estimated by the standard sodium bisulfite adducting method. See Table III.

Table - I

Weight of Lemongrass oil taken = 4.160 gm

Ratio of substance to adsorbant = 1:35

No.	Solvent	Vol. of solvent (ml)	Weight of substance (gm)	Method of Analysis	Remarks	Percentage
1.	Hexane	1000	0.255	TLC & GLC	Mixture of hydrocarbons	6.13
2.	98% hexane + 2% ether	1500	0.300	-do-	Geranyl acetate and carbonyl components other than citral	7.21
3.	95% hexane + 5% ether	2000	3.170	-do-	Citral	76.21
4.	100% ether	750	0.400	-do-	Mixture of Alcohols	9.62
	Total	5250	4.125			99.4

Table - II

Sl.No.	Sample	% of components				Percentage recovery
		Hydrocarbons	Carbonyl compounds other than citral geranyl acetate	Citral	Alcohols	
1.	Jammu RRL - 16	6.13	7.21	76.2	9.6	99
	Bhubaneswar					
2.	O.D. - 440	Traces	7.0	73.0	18.0	98.8
3.	O.D. - 19	2.062	6.184	67.64	23.763	98.6
4.	RRL - B	4.035	8.381	65	21.46	99.3
5.	RRL - 16	2.235	5.865	72.28	18.43	99.8
6.	S.D. - 68	1.0	8.0	71	19	99.0
7.	Sample - I	1.0	4.0	73	21	99.0
8.	Sample - II	5.0	4.0	83.5	7	99.5
9.	Sample - III	5.0	2.5	76.5	15	99.0
10.	Sample - IV	2.36	4.1	81	12	99.46

Table II contd.

Sl.No.	Sample	% of components				Percentage recovery
		Hydrocarbons	Carbonyl compounds other than citral geranyl acetate	Citral	Alcohols	
	Odakkali					
11.	O.D. - 440	1.5	2.0	90.00	5.0	98.5
12.	O.D. - 408	2.6	3.2	75.0	17.0	97.8
13.	O.D. - 19	0.3631	5.024	72.8	20.310	98.5
	Jorhat					
14.	RRL - J. No.1	3.84	8.63	68.85	17.1	99.42
15.	RRL - J.No.2	4.21	10.2	71.09	14.2	99.70
16.	RRL - J.No.3	2.0	3.055	72.33	21.10	98.45
17.	RRL - J.No.4	2.765	3.225	73.26	19.82	99.08
18.	RRL - J.No.5	3.368	5.855	69.97	18.93	98.123
19.	Sample from Bhuttan	5.0	8.25	67.98	17.75	98.98
20.	Commercial sample	7.0	7.63	70.25	13.35	98.25

N.B. Citral content decrease on storage of the oil

Table III contd.

Sl.No.	Samples	% of citral		Excess of citral by adducting
		Column Chromatography	Sodium bisulphite adducting	
	Jorhat			
11.	RRL - J. No.1	68.85	..	..
12.	RRL - J. No.2	71.09	..	..
13.	RRL - J. No.3	72.33	..	..
14.	RRL - J. No.4	73.26	..	..
15.	RRL - J. No.5	69.97	..	..
	Odakkali			
16.	O.D. - 440	90.00	94.0	4.0
17.	O.D. - 408	75.0	84.0	5.0
18.	O.D. - 19	72.8	76.0	3.2
19.	Commercial sample from Cochin	70.25	78.0	7.75
20.	Bhuttan	68.98	76.0	8.0

Table - III

Sl.No.	Samples	% of citral		Excess of citral by adducting
		Column Chromatography	Sodium bisulphite adducting	
1.	JAMMU - RRL - 16	76.2	84.0	7.8
	Bhubaneswar			
2.	O.D. - 440	73.0	80.0	7.0
3.	O.D. - 19	67.64	76.0	8.34
4.	RRL - B	65.0	72.0	7.0
5.	RRL - 16	72.28	75.0	2.72
6.	SD - 68	71.0	80.0	9.0
7.	Sample - I	73.0	78.0	5.0
8.	Sample - II	83.5	88.0	4.5
9.	Sample - III	76.5	79.0	2.5
10.	Sample - IV	81	86.0	5.0

5.3.2 Typical procedure for estimation of citral in Lemongrass oil by sodium bisulfite adducting[1]

10 cc of lemongrass oil taken in a Cassia flask (150 cc) was treated with 75 cc of freshly prepared saturated aqueous solution of sodium bisulfite. The adduct thus obtained after thorough shaking was made into a solution by immersing the flask in boiling water. A further addition of 25 cc of saturated bisulfite solution was made. The flask was then kept undisturbed to allow the unreacted oil to rise to the surface. After cooling the oil layer was brought upto the graduation mark by adding sodium bisulfite solution and the amount of unreacted oil measured. The citral content was calculated as follows:

Percentage of citral = 10 (10 - No. of cc of unreacted oil)

5.4 A new method of separation of citral from Lemongrass oil

5.4.1 Procedure

Silicagel (Sisco, Bombay - Meshsize 100-200) was used as such after activation at a temperature of 100°C for 1 hour. Solvent grade hexane and isopropanol (Merck India) were used as eluents after proper drying. Different ratios of adsorbant to silicagel were tried with varying elution rate for the quantitative separation of pure citral from lemongrass oil by column

Chromatography. The ratio of substance to adsorbant used in different experiments were 1:20, and 1:10. Separation was done on a 25 gm and 75 gm scale.

Fractions were collected in portions of 50 ml and analysed using TLC and GLC. Fractions having the same Rf value in TLC and same retention time in GLC were combined and solvent removed and weighed. When the minor components (Hydrocarbons, geranyl acetate and carbonyl compounds other than citral) were eluted out (as seen by TLC and GLC), polarity of the eluent was increased by adding isopropanol. When citral fractions were over (checked by TLC and GLC), the rest of the components (alcohols) were eluted out in a single lot. The purity of citral separated was checked by TLC and GLC Column elution details are given in Table IV, V, VI.

5.5 Total Analysis of Lemongrass oil

5.5.1 Separation of hydrocarbons from the first fraction obtained by column chromatography of lemongrass oil

The first fraction on GLC analysis was found to be a mixture of 3 hydrocarbons namely myrcene, limonene and dipentene.

It was chromatographed on silicagel (Sisco-Mesh 100-200) activated for 1 hour at 100°C with a ratio of substance to adsorbant 1:50. Fractions were collected

Table - IV

Weight of Lemongrass oil taken = 20.1500 gm

Ratio of substance to adsorbant used = 1:20

Rate of elution = 25 ml/minute

No.	Solvent	Vol. of solvent (ml)	Weight of substance (gm)	Method of Analysis	Remarks	Percentage
1.	Hexane	350	1.410	TLC & GLC	Mixture of hydrocarbons	7
2.	Hexane	1000	1.3702	-do-	Mixture of geranyl acetate and carbonyl compounds other than citral	6.8
3.	Hexane	800	0.645	-do-	Mixture of carbonyl compounds other than citral and citral	3.2
4.	99.5% hexane + 0.5% isopropanol	4500	14.105	-do-	Citral	70
5.	95% hexane + 5% isopropanol	1000	2.52	-do-	Mixture of alcohols	12.5
	Total	7650	20.052			99.5

Table - V

Weight of lemongrass oil taken = 24.890 gm

Ratio of substance to adsorbant = 1:10

Rate of elution = 9 ml/minute

No.	Solvent	Vol. of solvent (ml)	Weight of substance (gm)	Method of Analysis	Remarks	Percentage
1.	Hexane	150	1.74	TLC & GLC	Mixture of hydrocarbons	7
2.	Hexane	250	2.00	-do-	Mixture of geranyl acetate and carboxyl compounds other than citral	8
3.	99.5% hexane + 0.5% isopropanol	4700	17.92	-do-	Citral	72
4.	4.95% hexane + 5% isopropanol	1600	3.04	-do-	Mixture of alcohols	12.23
	Total	6700	24.70			99.23

Table - VI

Weight of Lemongrass oil taken = 75.00 gm

Ratio of substance to adsorbant = 1:10

Rate of elution = 12 ml/mt.

A glass column of 125cm x 5cm(ID) was used for the experiment.

Sl.No.	Solvent	Vol. of solvent (ml)	Weight of substance (gm)	Method of Analysis	Remarks	Percentage
1.	Hexane	3000	5.22	TLC & GLC	Hydrocarbons	7
2.	98% hexane + 2% isopropanol	500	6.01	-do-	Geranyl acetate and carbonyl compounds other than citral	8
3.	95% hexane + 5% isopropanol	1400	54.03	-do-	Citral	72
4.	75% hexane + 25% isopropanol	500	8.98	-do-	Alcohols	12
	Total	6400	74.24			99

in portions of 10 ml and checked by GLC. Fractions with same retention time in GLC were combined, solvent removed and weighed (See Table VII).

5.5.2 Separation of the components of the second fraction obtained by Column Chromatography of Lemongrass oil

The 2nd fraction was found to be a mixture of geranylacetate, citronellal, methyl heptenone and n-decyl aldehyde by GLC. This mixture was column Chromatographed using silicagel, (Sisco Meshsize 100-200), after activating for 1 hour at 100°C, as adsorbant and a mixture of 99% hexane and 1% isopropanol as eluents. The ratio of adsorbant to substance used was 1:50. Fractions were collected in portions of 25 ml and checked by TLC and GLC. Fractions having same Rf value in TLC and same retention time in GLC were combined, solvent removed and weighed(See Table VIII).

5.5.3 Separation of alcohols obtained by column chromatography of lemongrass oil

The last fraction was found to be a mixture of alcohols namely geraniol, nerol, linalool, methyl heptenol and citronellol by GLC. The mixture was column chromatographed on silicagel (Sisco-meshsize 100-200)

Table - VII

Weight of hydrocarbons taken = 2.0 gm

Ratio of substance to adsorbant (silicagel - Sisco-Meshsize 100-200) = 1:50

Rate of elution = 5 ml/minute

No.	Solvent	Vol. of Solvent (ml)	Weight of Substance (gm)	Method of Analysis	Remarks	Percentage
1.	Hexane	2500	1.286	TLC & GLC	Myrcene	64.3
2.	Hexane	1500	0.714	-do-	Mixture of limonene & dipentene	35.7
	Total	4000	2.000			100

% of myrcene present in Lemongrass oil = 4.5%

% of Limonene + dipentene present in lemongrass oil = 2.5%

Table - VIII

Weight of 2nd fraction taken = 2.00 gm

Ratio of substance to adsorbant (Silicagel-Sisco-meshsize 100-200) = 1:50

Rate of elution = 5 ml/minute

Sl.No.	Solvent	Vol. of Solvent (ml)	Weight of Substance (gm)	Method of Analysis	Remarks	Percentage
1.	99% hexane + 1% isopropanol	1200	0.236	TLC & GLC	n-decyl aldehyde + citronellal	11.8
2.	99% hexane + 1% isopropanol	2250	1.400	-do-	Geranyl acetate	70.0
3.	99% hexane + 1% isopropanol	760	0.36	-do-	Methyl heptenone	18
	Total	4210	1.996			99.8

Percentage of citronellal + n-denyl aldehyde present in lemongrass oil = 0.8%

Percentage of geranyl acetate present in Lemongrass oil = 5.6%

Percentage of methyl heptenone present in Lemongrass oil = 1.44%

after activation for 1 hour at 100°C. Fractions were collected in portions of 25 ml and fractions with same retention time in GLC were combined, solvent removed and weighed. Results are given in Table IX

5.6 Separation of components of non-citral portion of Lemongrass oil by Column Chromatography

Non-citral portion of lemongrass oil (citral separated by adducting with sodium bisulfite)[8] was column chromatographed using silicagel (BDH-Mesh 60-120), after activation for 1 hour at 100°C, as adsorbant and hexane and ether as eluents after proper drying. The experiment was done on a (55x3 cm) glass column and a constant elution rate maintained during the whole period of elution. Fractions were collected in portions of 50 ml and checked by TLC and GLC. Fractions with same Rf value in TLC and same retention time in GLC were combined, solvent removed and weighed. Hexane was used for collecting the hydrocarbons. When all the hydrocarbons were over (as seen by TLC and GLC) the polarity of the solvent is slightly increased by using a mixture of 98% hexane and 2% ether as solvent. When citral started coming the polarity was further increased to 95% hexane and 5% ether. When all the citral present were collected,

Table - IX

Weight of alcohols taken = 2.00 gm

Ratio of substance to adsorbant (Silicagel-Sisco-Meshsize 100-200) = 1:50

Rate of elution = 5 m/minute

Sl.No.	Solvent	Vol. of Solvent (ml)	Weight of Substance (gm)	Method of Analysis	Remarks	Percentage
1.	95% hexane + 5% isopropanol	275	0.1136	GLC & TLC	Methyl heptenol	5.68
2.	95% hexane + 5% isopropanol	750	0.486	-do-	Linalool	24.3
3.	95% hexane + 5% isopropanol	1500	1.398	-do-	Geraniol+ nerol + Citronellol	69.9
	Total	2525	1.9976			99.8

Percentage of methyl heptenol present in Lemongrass oil = 0.7%

Percentage of linalool present in Lemongrass oil = 3.0%

Percentage of geraniol + nerol + citronellol present in Lemongrass oil = 8.63%

the rest (mixture of alcohols) was eluted in a single lot using 100% ether.(See Table X).

5.7 Separation of components of ionone tops

Lemongrass oil was condensed with acetone in basic media[22] and then vacuum distilled. The first fraction collected (ionone tops) was then column chromatographed using silicagel (BDH Mesh 60-120), after activating for 1 hour at 100°C, as adsorbant and hexane and ether as eluents. The experiment was done on a (55 x 3 cm ID) glass column. A constant elution rate was maintained throughout the chromatography. A ratio of 1:35 for substance to adsorbant was used for the experiment.

Fractions were collected in portions of 50 ml and analysed by TLC and GLC. Fractions having same Rf value in TLC and same retention time in GLC were combined, solvent removed and weight taken. Hexane was used as the eluent for eluting the hydrocarbons when the hydrocarbon fractions were over (as seen from TLC and GLC) polarity of eluent was increased by adding a mixture of 2% ether and 98% hexane. When citral started coming (as seen from TLC and GLC) polarity of eluent was further increased from 2% ether to 5% ether. After collecting all the citral containing fractions, the

Table - X

Weight of non-citral portion taken = 4.080 gm

Ratio of substance to adsorbant = 1:35

Sl.No.	Solvent	Vol. of Solvent (ml)	Weight of Substance (gm)	Method of Analysis	Remarks	Percentage
1.	Hexane	1000	1.069	TLC & GLC	Mixture of hydrocarbons	26.22
2.	98% hexane + 2% ether	1750	0.77	-do-	Mixture of geranyl acetate and carbonyl compounds other than citral	19.03
3.	95% hexane + 5% ether	750	0.375	-do-	Citral	9.212
4.	100% ether	2000	1.75	-do-	Mixture of alcohols	43.07
	Total	5500	3.964			97.53

rest (alcohols) was collected in a single lot using 100% ether. Results are given in Table XI.

5.8 Separation of the cis trans isomers of citral

99+ % pure citral separated from lemongrass oil using column chromatography was again chromatographed with a view to obtain pure geranial and pure neral.

Silicagel (BDH Mesh 60-120), after activation for 1 hour at 110°C, was used for the chromatographic studies solvent grade hexane and ether were used as eluents after proper drying. Different ratios of the substance to adsorbant and different elution rates were tried for the experiment. Glass columns of (110 x 25 mm ID) were used. Rate of elution was maintained at 38 ml/minute. With a ratio of 1:35, a reversal of the ratio of neral to geranial was found, on GLC analysis, in the fractions collected (See Table XII).

Fractions were collected in portions of 25 ml and analysed by GLC. Fractions with same retention time in GLC were combined, solvent removed and weighed.

With a ratio of substance to adsorbant 1:75, it was possible to get 13% pure neral and 45% pure

Table - XI

Weight of ionone tops taken = 6.11 gm

Sl.No.	Solvent	Vol. of Solvent (ml)	Weight of Substance (gm)	Method of Analysis	Remarks	Percentage
1.	Hexane	1500	0.730	TLC & GLC	Mixture of hydrocarbons	12
2.	98% hexane + 2% ether	1250	0.6535	-do-	Mixture of geranyl acetate and carbonyl compounds other than citral	1.07
3.	95% hexane + 5% ether	500	0.050	-do-	Citral	0.81
4.	100% ether	1750	4.420	-do-	Mixture of alcohols	72.35
	Total	5000	5.8535			95.9

Table - XII

Weight of citral taken = 2 gm

Ratio of substance to adsorbant (Silicagel BDH Meshsize 60-120) = 1:35

Rate of elution = 5 ml/minute

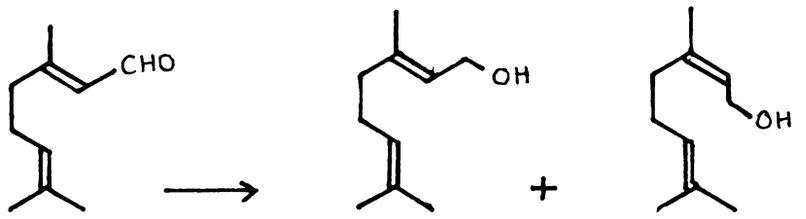
Sl.No.	Solvent	Vol. of Solvent (ml)	Weight of Substance (gm)	Method of Analysis	Remarks	Percentage
1.	98% hexane + 2% ether	750	0.38	GLC	60% neral+ 40% geranial	19
2.	98% hexane + 2% ether	1250	0.70	"	50% neral + 50% geranial	35
3.	98% hexane + 2% ether	2000	0.88	"	20% neral + 80% geranial	44
	Total	4000	1.96			98

geranial and the rest mixture of neral and geranial with varying amounts of the two isomers (See Table XIII).

Finally with a ratio of substance to adsorbant 1:100, it was possible to get 24% pure neral and 33% pure geranial (See Table XIV). Using silicagel (Sisco Meshsize 100-200) as adsorbant and an elution rate of 15 ml/minute it was found possible to get 5% neral, 16% geranial and the rest a mixture of neral and geranial with varying ratios, using a ratio of substance to adsorbant 1:10 (See Table XV).

5.9 Separation of nerol and geraniol by Column Chromatography

99+% pure citral (1) separated from lemongrass oil was converted to the corresponding unsaturated alcohols nerol (2) and geraniol (3) by reduction with Aluminium isopropoxide in anhydrous isopropanol. The reaction can be represented as



(1) (3) (2)

Table - XIII

Weight of citral taken = 3.7300 gm

Ratio of substance to adsorbant (Silicagel BDH Mesh-size 60-120) used = 1:75

Rate of elution = 22 ml/minute

Sl.No.	Solvent	Vol. of Solvent (ml)	Weight of Substance (gm)	Method of Analysis	Remarks	Percentage
1.	98% hexane + 2% ether	750	0.485	GLC	Pure neral	13
2.	98% hexane + 2% ether	1250	1.53	-do-	Neral + Geranial	41
3.	98% hexane + 2% ether	1750	1.68	-do-	Pure Geranial	45
	Total	3750	3.695			99

Table - XIV

Weight of citral taken = 2.73

Ratio of substance to adsorbant (Silicagel-BDH-Meshsize 60-120) = 1:100

Rate of elution = 38 ml/minute

Sl.No.	Solvent	Vol. of Solvent (ml)	Weight of substance (gm)	Method of Analysis	Remarks	Percentage
1.	98% hexane + 2% ether	1500	0.660	GLC	Pure neral	24
2.	98% hexane + 2% ether	1250	1.130	-do-	Neral + Geranial	42
3.	95% hexane + 5% ether	1750	0.915	-do-	Pure Geranial	33
	Total	6500	2.705			99

Table - XV

Weight of citral taken = 9.600 gm

Ratio of substance to Adsorbant (Silicagel-Sisco Meshsize 100-200, activated for 1 hour at 10°C) = 1:20

Rate of elution = 13 ml/minute

Sl.No.	Solvent	Vol. of Solvent (ml)	Weight of Substance (gm)	Method of Analysis	Remarks	Percentage
1.	98% hexane + 2% ether	1000	0.48	GLC	Pure neral	5
2.	98% hexane + 2% ether	375	0.24	-do-	90% neral + 10% geranial	2.5
3.	98% hexane + 2% ether	1000	0.55	-do-	60% neral + 40% geranial	5.7
4.	98% hexane + 5% ether	2000	0.45	-do-	5% neral + 50% geranial	4.7
5.	95% hexane + 5% ether	2500	1.58	-do-	40% neral + 60% geranial	16.46
6.	90% hexane + 10% ether	550	0.77	-do-	35% neral + 65% geranial	8
7.	90% hexane + 10% ether	2500	3.94	-do-	30% neral + 70% geranial	41.04
8.	100% ether	250	1.55	-do-	Pure geranial	16.15
	Total	10175	9.56			99.5

5.9.1 Procedure

In a 500 cc RB flask, were placed 46 gm(0.3 mol) of citral and 300 cc of 1 M solution of aluminium isopropoxide in anhydrous isopropanol. A short reflux condenser was attached to the flask, without running water through the cooling jacket. Then another water cooled condenser was also attached so as to distill the acetone formed in the reaction with the aid of a distilling head. The contents of the flask were refluxed on a water bath. The distillate (acetone) were collected at a slow rate. The presence of acetone in the distillate was tested with 2,4-dinitrophenylhydrazene reagent.

When the acetone test became negative water was then passed through the upright condenser and total reflux was maintained for 5 minutes. The water was again removed from the reflux condenser and the first few drops of the distillate were tested for acetone when the negative test was obtained, excess isopropyl alcohol was recovered under slightly reduced pressure. The cooled residue was hydrolysed with cold dilute hydrochloric acid and the cooled suspension was mixed well by swirling to complete the hydrolysis. The mixture was extracted with ether, the ether layer washed with water and dried using anhydrous sodium sulphate. Ether removed and the product analysed

using TLC.

The analysis showed the possibility of some side reaction taking place. The side product being hydrocarbons.

The reduction was repeated three times to confirm the formation of side products. The product was analysed using column chromatography and also the alcohol content determined by acetylation. Results obtained are given in Table XVI.

5.9.2 Typical procedure for acetylation of the alcohols

The reduction product obtained was acetylated using acetic anhydride and sodium acetate. It was then hydrolysed using a known amount of 0.5 N NaOH and the excess alkali was determined by back titration with 0.5 N HCl. The volume of alkali consumed for the hydrolysis of the ester formed to alcohol was calculated.

$$\% \text{ of alcohol} = \frac{am}{20(S - 0.021 \times a)}$$

where a is the volume of 0.5 N NaOH used for the hydrolysis of the acetate formed, m is the molecular weight of the alcohol and S is the weight of ester taken for hydrolysis.

Trial was conducted on the separation of the reduction product obtained with a view to separate

Table - XVI

Analysis of the Reduction products of Citral

Sl.No.	Percentage of Alcohol By Column Chromatography	By Acetylation	Percentage of side product
1.	69.36	71.94	21.4
2.	71.94	72.0	20.66
3.	71.23	72.0	19.62

nerol and geraniol from the mixture. The product was separated into hydrocarbons (20%) pure nerol (5%), mixture of nerol and geraniol (48.2%) and pure geraniol (25%). Results are given in Table XVII

Table - XVII

Weight of reduced citral taken = 13.10 gm

Ratio of substance to adsorbant used = 1:12

Sl.No.	Solvent	Vol. of Solvent (ml)	Weight of substance (gm)	Method of Analysis	Remarks	Per-cent-age
1.	Hexane	250	2.620	TLC & GLC	Hydrocarbons	20
2.	Hexane	100	0.655	-do-	Nerol	5
3.	95% hexane + 5% ether	750	6.3142	-do-	Nerol + geraniol	48.2
4.	90% hexane + 10% ether	500	3.275	-do-	Geraniol	25
	Total	1600	12.86			98.2

S E C T I O N B

INDUSTRIAL USES FOR THE COMPONENTS OF LEMONGRASS OIL

C H A P T E R VI

INTRODUCTION

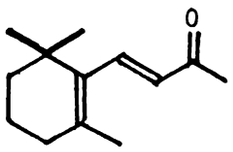
6. New Industrial uses for the components of Lemongrass oil

6.1. Introduction

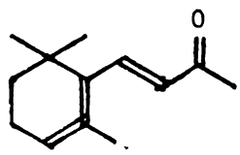
6.1.1 Novel Synthesis of Ionones

The discovery of ionones was undoubtedly a milestone in the development of synthetic perfumery chemicals. One effect of this discovery was that the perfumer started appreciating the value of synthetics and was ready to accept them as useful adjuncts in place of natural fragrances.

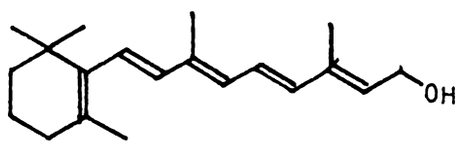
Until 1927, when Penfold[1] reported the presence of β-ionone in Boronia Megastigma N., there was no indication of its occurrence in nature. Since then about a dozen sources of α and β-ionone (1) and (2) have been described in the literature[1 a]. In addition, it is known that both alpha and beta ionones occur in small quantities in the flavour components of most fruits, and in relatively large quantities in berry-type fruits. The violet odour of some palm oils has been attributed to the breakdown of β-Carotene into ionones[2].



(2)



(1)



(3)

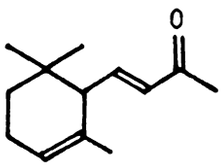
As the value of ionone and its homologs gradually became recognized, perfumers began to use them in practically all types of compositions. Although the ionones are a group of chemicals having a violet odour, their use is not confined to this fragrance. In general the incorporation of ionones in fragrance formulas imparts a pleasing sweetness which adds warmth and depth to the creation.

The manufacture of synthetic Vitamin A (3) has created a strong demand for very large quantities of β-ionone. Since β-ionone is the key intermediate in many of the approaches in the manufacture of Vitamin A, much work has been done in exploring

different approaches to the synthesis of citral and β-ionone. Pseudoionones also find use in the synthesis of phytol, which in turn is used for the synthesis of Vitamin E.

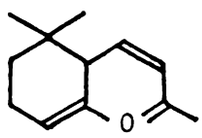
The importance of ionones is because of the very large amounts consumed annually by the perfume, soap, cosmetic and Vitamin industries. The consumption of methyl ionones in perfumery far exceeds that of α and β-ionones. While the ionones have good odour stability, they sometimes cause yellowing especially when used in high concentrations.

One can obtain many isomers of ionone and methyl ionone. For example, α-ionone having an optically active carbon atom can exist in d (+) and l (-) forms. In addition, having an exocyclic double bond, it can exist in the cis and trans forms, giving four α-ionones (4-7). β-ionone has no chirality so it can exist only in the cis and trans forms (8 & 9). γ-ionone has an optically active atom and also an exocyclic double bond and can therefore exist in four forms (10-13). These make a total of 10 different ionones.



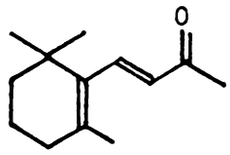
(4)-d-trans

(5)-l-trans

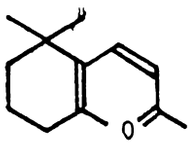


(6)-d-cis

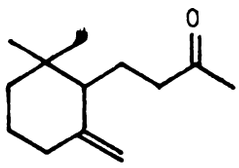
(7)-l-cis



(8)-trans

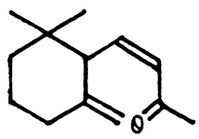


(9)-cis



(10)-d-trans

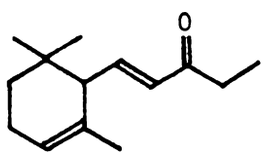
(11)-l-trans



(12)-l-cis

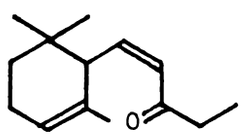
(13)-d-cis

Condensation of methyl ethyl ketone and citral, followed by cyclisation leads to the formation of 20 isomers of methyl ionone (14-33,



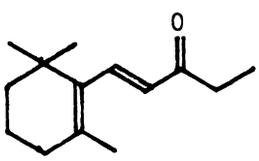
(14)-d-trans

(15)-l-trans

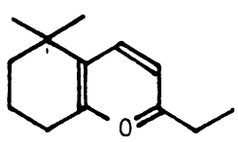


(16)-d-cis

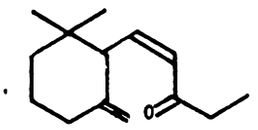
(17)-l-cis



(18)-trans

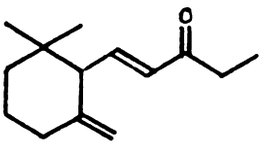


(19)-cis



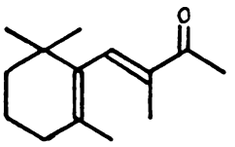
(20)-d-cis

(21)-l-cis

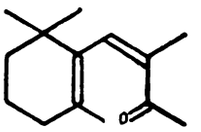


(22)-d-trans

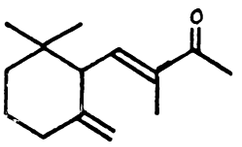
(23)-l-trans



(28)-trans

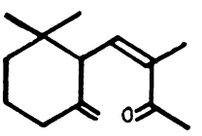


(29)-cis



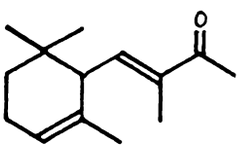
(30)-d-trans

(31)-l-trans



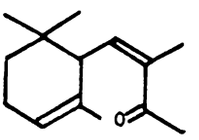
(32)-d-cis

(33)-l-cis



(24)-d-trans

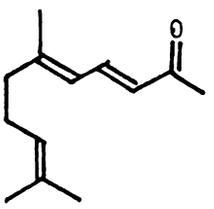
(25)-l-trans



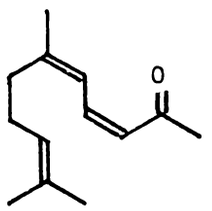
(26)-d-cis

(27)-l-cis

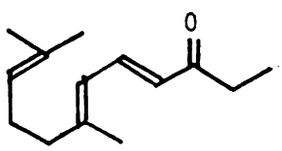
Because of their great use in industries, new and easy methods for the conversion of citral to pseudoionones (34, 35), ionones and methyl-pseudo-ionones (36, 37) were planned and easier and efficient methods for their synthesis were achieved.



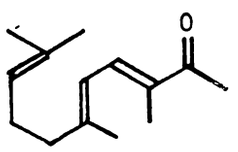
(34)



(35)



(36)



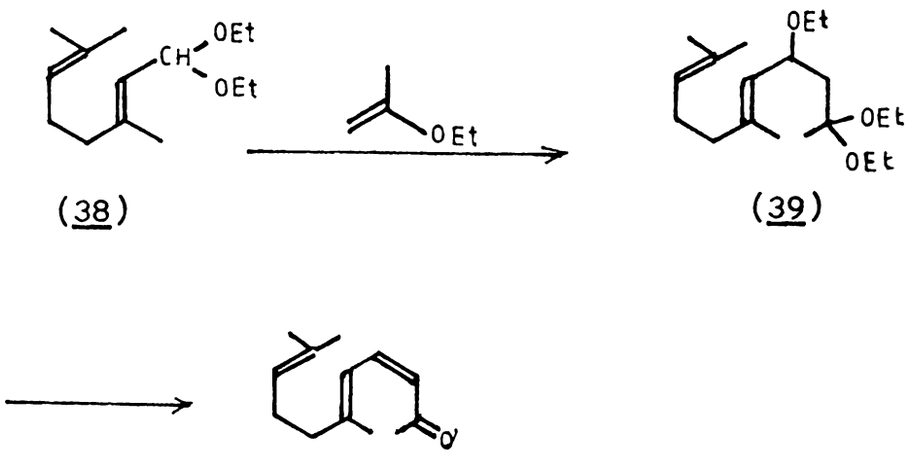
(37)

6.1.2 Pseudoionones

Various methods were reported in the literature for the synthesis of pseudoionones (34, 35)[3-23] including the condensation of citral with acetone in presence of bases like alkalimetal hydroxides[9-13].

Condensation of citral acetal (38) with 2-ethoxy-propane yields the triethoxy derivative of pseudoionone (39), which can be converted to pseudoionones (34,35)[17] as in Scheme I [39].

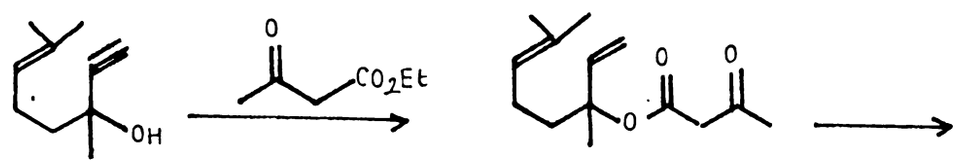
Scheme - I



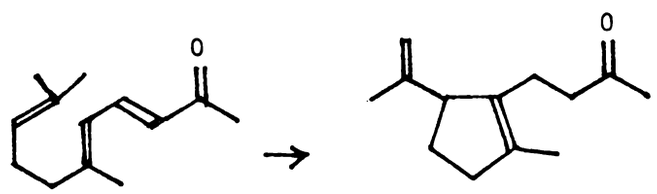
(38) (39)

It was reported that in the formation of pseudoionones (34,35) by the pyrolysis of dehydrolinalyl acetoacetate (40), a ketone is also formed which is different from pseudoionones. It was found to be a cyclopentane derivative (41).(Scheme II).

Scheme II



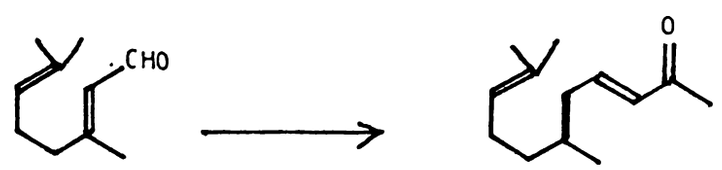
(40)



(41)

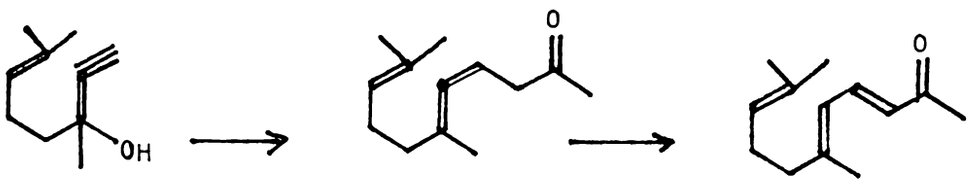
In the condensations of citral with acetone using alkali (Scheme III), the excess alkali has to be neutralised and hence there are chances of polymerisation of the highly conjugated pseudoionones which may explain the lower yields generally obtained.

Scheme III



Very pure pseudoionones were obtained when isopropnenyl ethyl ether was treated with dehydro-linalool (42) in the presence of traces of alkali[24] (Schéme IV).

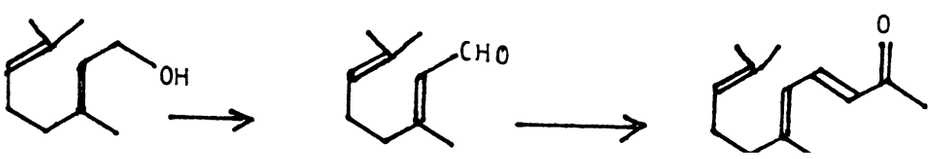
Scheme IV



(42)

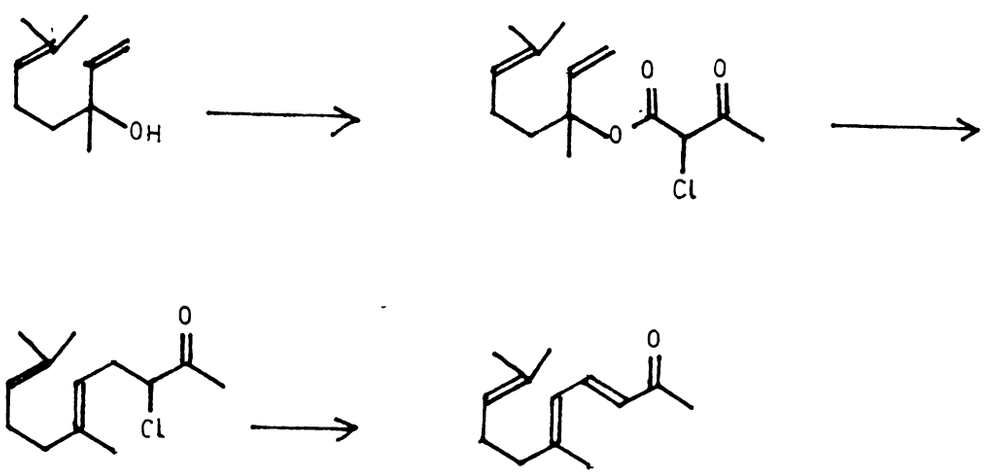
By reacting geraniol with aluminium alkaloxides in acetone, good yields of pseudoionone can be obtained[25] (Scheme V).

Scheme V



Pseudoionone was also prepared from linalool and chloroacetoacetic ester[26] (Scheme VI).

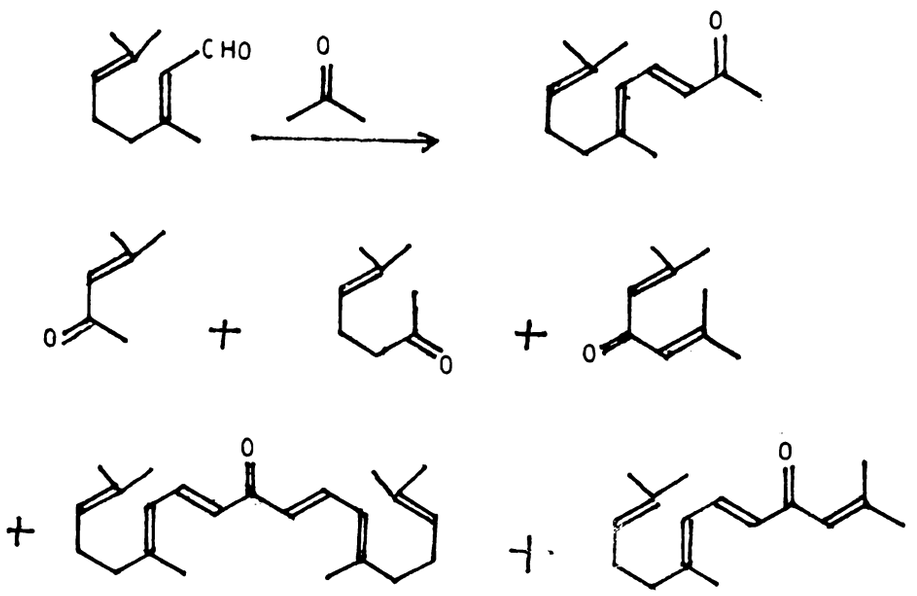
Scheme VI



Many by-products were formed when citral is condensed with ketones. In the simplest case of citral and acetone, it is possible to get mesityl oxide, and polymers of citral from self condensation, as well as products from the condensation of one molecule of acetone with two of citral and of citral

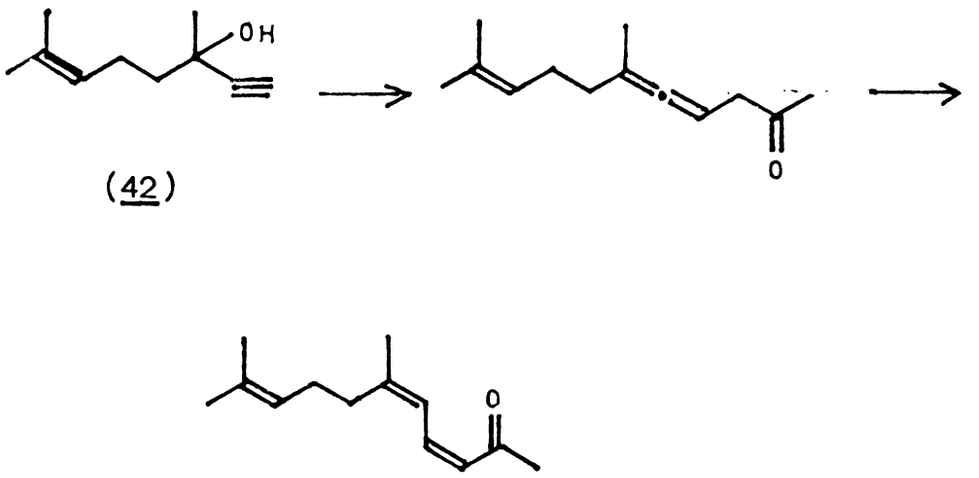
with mesityl oxide and higher homologs. These can explain the lower yields of pseudoionones generally obtained[27] (Scheme VII).

Scheme VII



Alumina was reported to catalyse reactions like reduction, hydride transfer reactions like disproportionation and dehydration[28-29], epoxide opening[30], and cis isomerisation of β-ketoallenes to conjugated cisdienones[31]. The cisisomer of pseudoionones was prepared in 93% purity by the alumina catalysed isomerisation of the allene obtained from dehydrolinalool (42) and dl-isopropyl methyl ether[31-32] (Scheme VIII).

Scheme VIII



(42)

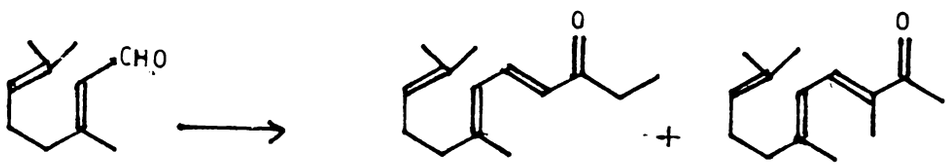
Since alumina is known to catalyse dehydrations, the usefulness of basic alumina as the condensation catalyst for the condensation of citral with acetone was studied. The results obtained were promising. Different ratios of citral, acetone and alumina were tried and the best ratio was found to be 1:40:20. Using basic alumina as the condensation catalyst, pseudoionones of 99% purity (checked by GLC) were obtained in 93% yield. This method is a simple method requiring no neutralisation of excess alkali as in the case of many other methods. Moreover

alumina and excess acetone can be recovered and reused.

6.1.3 Methyl Pseudoionones

Synthesis of methyl pseudoionones (36) and (37) by the condensation of citral and ethyl methyl ketone is more complex than the synthesis of pseudoionones (Scheme IX). The reaction of the aldehyde group of citral with either the methyl or methylene group of ethyl methyl ketone gives n- and isomethyl pseudoionones (36) and (37), each of which being capable of occuring as four cis trans isomers. Since these isomers possess different odours, their separation to individual ionones has practical value.

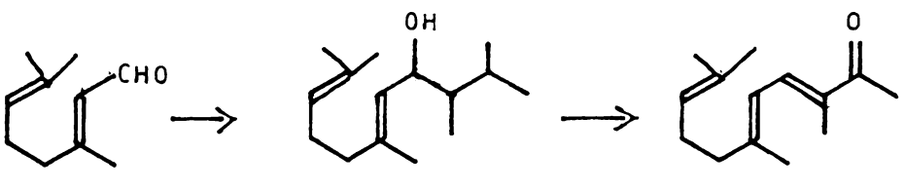
Scheme IX



(36) (37)

various methods were reported for the condensation in literature[33-37]. Treatment of the Grignard of 3-chlorobutanone-2 with citral produced hydroxydihydro-pseudo isomethyl ionone which on dehydration gave pseudoisomethyl ionone (Scheme X). The ratio of the major isomers in the mixture depends on the condensation catalyst and the reaction conditions. In the presence of common alkaline catalysts, the normal isomers were formed preferentially. It was observed that strongly alkaline bases such as quaternary ammonium salts favour the formation of isomethyl pseudoionones[38].

Scheme X

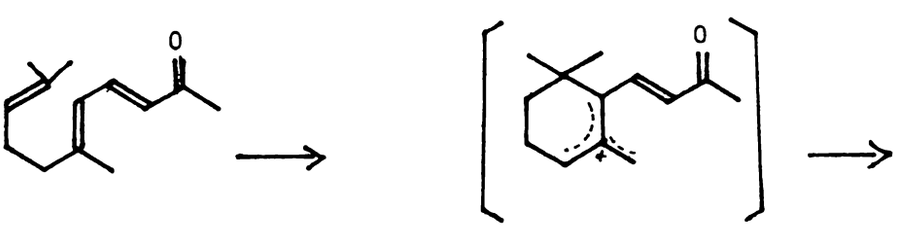


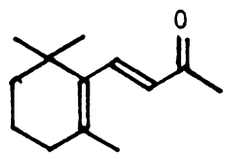
Since it was found possible to prepare pseudoionones from citral and acetone using basic alumina as the condensation catalyst, trial was made for the preparation methyl pseudoionone in a similar way. Thus 95% conversion of citral to methyl pseudoionone was achieved in 5 hours using citral, ethylmethyl ketone and alumina in the ratio of 1:40:20 under reflux. Here also the excess ethyl methyl ketone and alumina can be recovered and reused.

6.1.4 Cyclisation of Pseudoionones

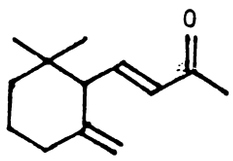
Cyclisation of pseudoionones with strong acids like sulfuric acid gives mainly β-ionone[39], whereas with mild acids like formic or phosphoric acids yields mainly the α-isomer[40]. The mechanism of cyclisation has been investigated[41] although more attention has been given to the practical aspects of the problem (Scheme XI).

Scheme XI

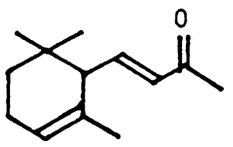




(2) +



(12) +

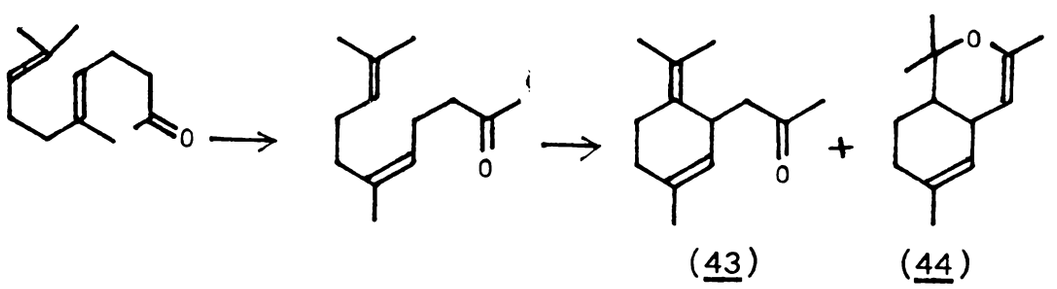


(1)

Using 100% sulfuric acid, the cyclisation of pseudoionones were studied over a range of temperatures[42]. It was found that at -60°C, only α-ionone is formed and at -40°C, either of the 3 isomers may predominate, depending upon the duration of the reaction. At higher temperatures, the proportion of β-ionone increases and at 10°C the product is almost all β-ionone. It thus appears that in concentrated sulfuric acid, α-ionone, isomerises to β-form. No isomerisation takes place with phosphoric acid or with 60% sulfuric acid. These results were substantiated in an American patent[43] where the advantages of using hexane as a solvent were stressed. Other patents describe the use of dl-isopropyl ether[44] or even ethyl ether[45] along with concentrated H_2SO_4 to

obtain high yields of β-ionone. Using a mixture of sulfuric and acetic acids, Naves and Ardizio[46] studied the various factors in the cyclisation of pseudoionones and found that the β-ionone proportions increased with a rise in the percentage of sulfuric acid. It appears that cyclisation product is not dependent on whether the starting material is cis, trans or a mixture of pseudoionones since all give the same end product under identical cyclisation conditions[47]. Cyclisation was also carried out using BF_3 at room temperature or lower[48]. After a detailed study of the problem of cyclisation, Naves and his co-workers[49] came to the conclusion that cyclisation reagent determines the nature of the product. This sulfuric, formic or phosphoric acids give a mixture of α-,β and γ -ionones, Lewis acids such as aluminium, ferric or Zinc chlorides give not only the ionones but also menthanic cyclisation products[49] and BF_3 gives mainly the 3 ionones[49] (Scheme XII).

Scheme XII



(43) (44)

Ionones were also prepared by cyclisation of the enol acetates of pseudoionones[50]. β-ionone of about 95% purity was prepared in 82% yield by using concentrated sulfuric acid and ethyl acetate at $5^{o}C$[14]. β-ionone was also reported to be formed in 57% yield by the cyclisation of pseudo-ionone using electrogenerated acid[51].

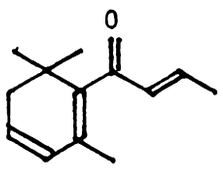
Since basic alumina was found to act as an effective catalyst in the pseudoionones, it was planned to do the cyclisation of pseudoionones to ionones using acidic alumina as the cyclisation catalyst. Different ratios of the substance, alumina and solvent (ethyl acetate) were tried at varying temperatures. But no cyclisation took place.

Acidic alumina soaked in p-toluene sulfonic acid was then tried for the cyclisation. Different percentages of the solutions of p-toluene sulfonic acid in ethylacetate were used (5%, 10%, 15% and 20%). Using 20% p-toluenesulfonic acid and taking the reagents pseudoionone; acidic alumina; 20% p-toluene sulfonic acid solution in ethyl acetate, in the ratio

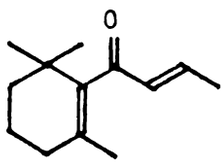
of 1:20:40, 98% conversion of pseudoionones to ionones occured in 15-30 minutes. In the mixture of ionones obtained β-ionone was formed in very small percentage α-isomer being the major isomer. Ratio of ionones was found to be 54:37:6.5. The present method can be considered as a very simple one compared to other existing methods for the cyclisation of pseudoionones. One of the advantages is that this cyclisation can be done at room temperature and the time required is reduced to 30 minutes. But by this method α-and γ-ionones are formed in substantial quantities and β-ionone only in small amounts. However it is possible to convert α and γ-isomers to the β-isomer by standard methods.

6.2 A new approach for the Synthesis of β-Damascone

In recent years the isolation of damascenone(45) and β-damascone (46) from several plants has stimulated considerable interest because of their powerful and pleasant fragrance. The damascones are nor-isoprenoidic natural products with valuable fragrance and flavour properties[52], and are in fact ionone isomers.



(45)

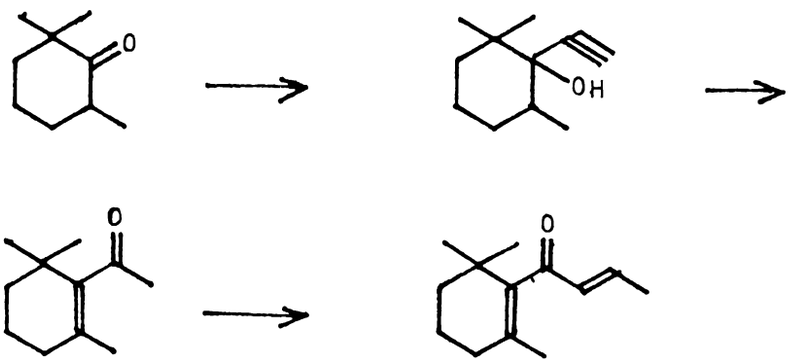


(46)

β-Damascone (46), a member of this small group of compounds has been detected in the Burley[53], Greek[54], Virginia[55] and oriental[56] brands of tobacco as well as in tea[57] and rose[58] oils. Both β-Damascenone (45) and β-Damascone (46) play a very important role in producing the characteristic odour of Otto of rose. Both of them are also reported to be organolyptic property improvers. The damascones are used in perfume compositions, in particular in rose perfumes and in flavour compositions. Due to their important odour properties, many elegant synthesis have been reported by various authors. Since these analogues including β-Damascone are very useful in perfumery fields and are not available from natural sources in sufficient quantities much effort on their synthesis have been devoted. IFF Chemists have developed a practical synthesis of β-Damascone[59] (46)

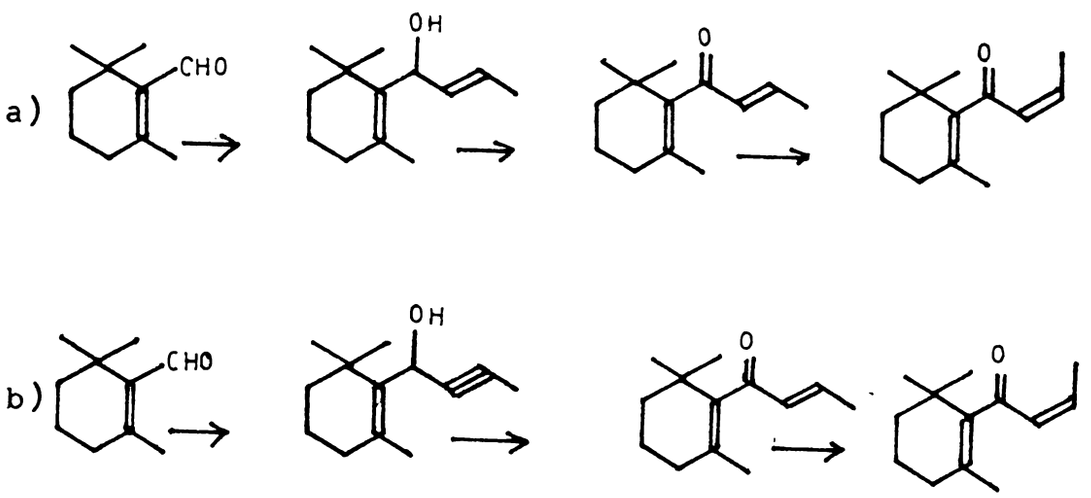
starting from 2,2,6 trimethyl cyclohexanone (Scheme XIII)

Scheme XIII

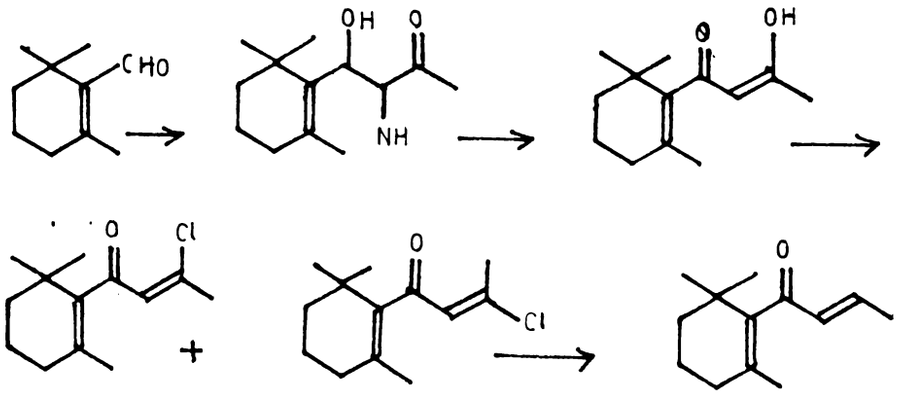


Synthesis of β-Damascone (46) starting from β-cyclocitral was also reported[60,61] (Scheme XIV and XV).

Scheme XIV

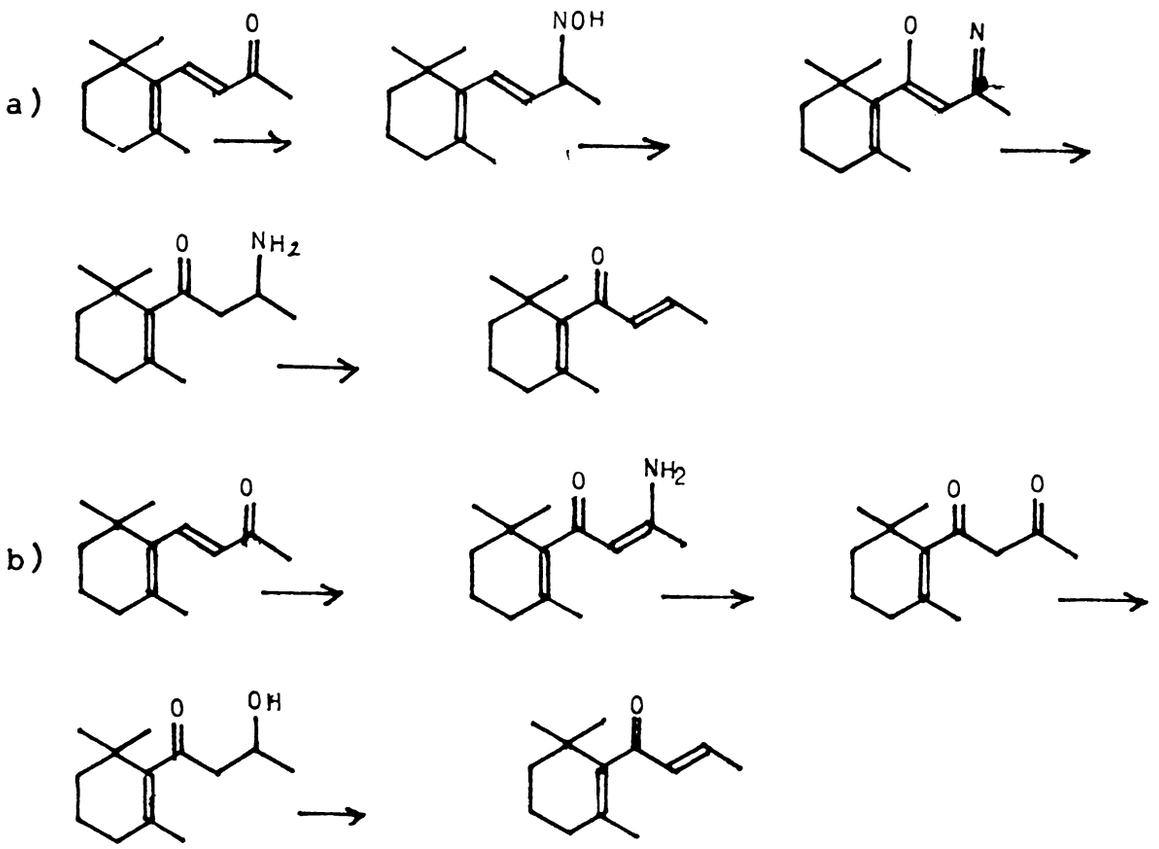


Scheme XV



Synthesis of β-Damascone (46) starting from β-ionone[62] was also reported (Scheme XVI).

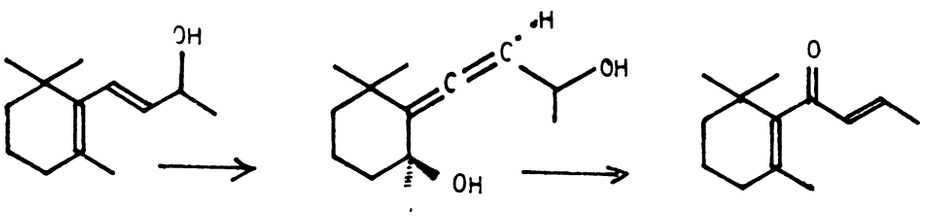
Scheme XVI



β-Damascone was also reported to be synthesized from cyclogeranic acid methyl ester[63] and 2,6,6-trimethyl cyclohexanone[64,65].

β-Damascone was prepared from β-ionol[66] through its allene (Scheme XVII).

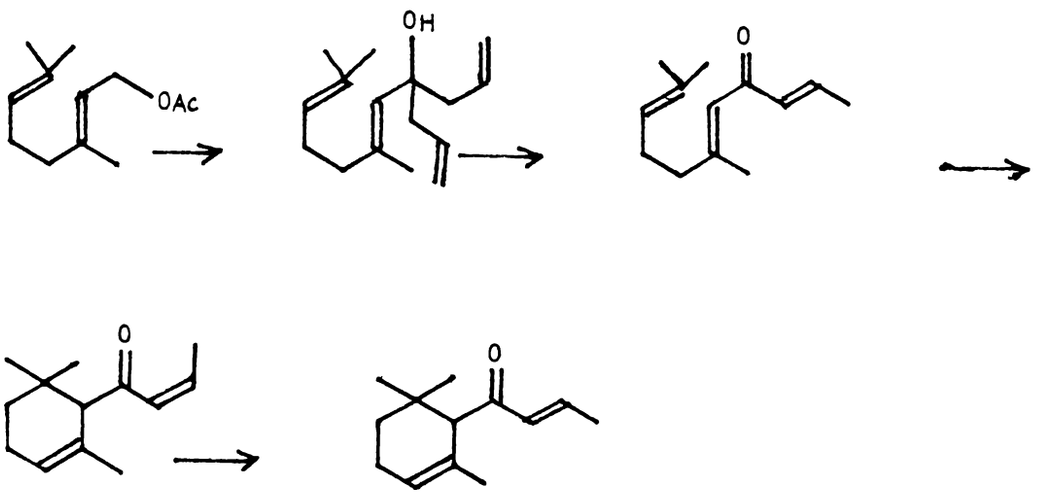
Scheme XVII



A novel synthesis of 3-hydroxy β-Damascone and β-Damascenone was reported by T.Kitahara, et al[67] via Diels Alder reaction.

Damascones were also synthesized by the acid catalysed cyclisation of pseudodamascones[68]. In this cyclisation in contrast to ionones, strong acids (sulfuric acid) favours the formation of the isomer[68] (Scheme XVIII).

Scheme XVIII

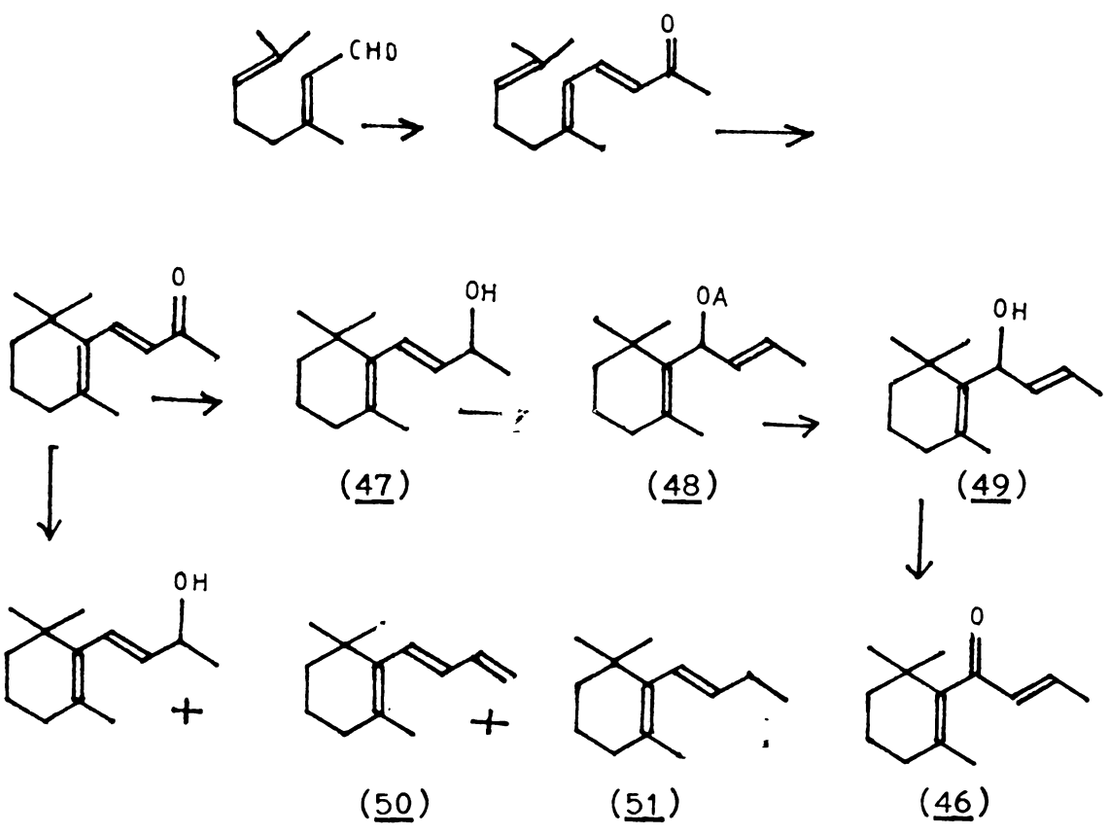


Since the readily available β-ionone represent an ideal raw material for the synthesis of β-Damascone and since β-Damascone was already prepared from β-ionone in four steps[62], it was planned to

transform β-ionone to β-damascone via an easier route. β-ionone was already prepared from citral, the main component of lemongrass oil.

Synthesis of β-Damascone starting from citral was achieved as given in (Scheme XIX).

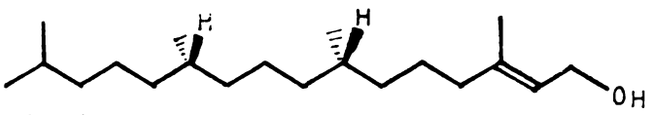
Scheme XIX



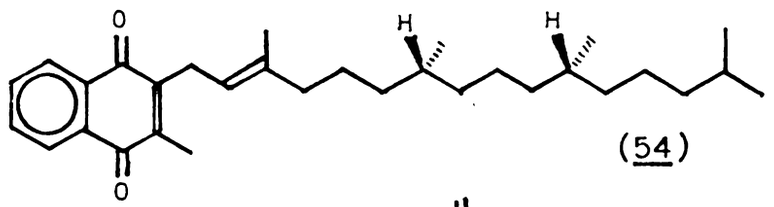
Citral was separated from lemongrass oil by column chromatography[69] and then condensed with acetone in presence of basic alumina to get pseudoionones[70]. It was then cyclised in ethyl acetate using concentrated sulfuric acid to get β-ionone[51]. β-ionone was then reduced by aluminium isopropoxide in anhydrous isopropanol. But it was found that β-ionol (47) was formed only in 28% and the rest being hydrocarbons. The crude product was then separated by column chromatography on silicagel and characterised. It was found that 65% of the reduction product was a mixture of (50) and (51). Hence the reduction was conducted using sodium borohydride in anhydrous methanol. By this method β-ionol (47) was obtained in high yields. No side product was formed. Acetylation of β-ionol was conducted at milder conditions by mixing the alcohol with a few drops of pyridine and excess acetic anhydride and keeping at room temperature for 24 hours. Near quantitative conversion of the alcohol to the allylically rearranged acetate (48) occured. Mild hydrolysis by stirring with methanolic potassium hydroxide at room temperature gave iso-β-ionol (49) in 90% yield. Iso-β-ionol was then oxidised using Jone's reagent to give β-Damascone (46) which was then purified by chromatography and characterised by spectral data.

6.3 A new approach to the Synthesis of Phytol

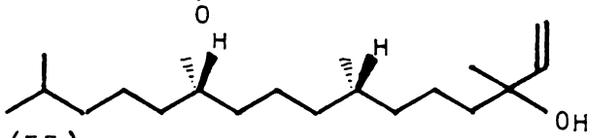
Natural phytol (52) an optically active C_{20} terpenic alcohol,which forms 34.25% the weight of chlorophyll-a,finds many industrial applications. The most important use of phytol and its derivatives are in the synthesis of tocopherols (53) and Vitamin K_1 (54).



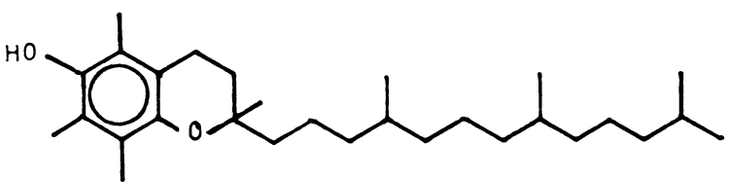
(52)



(54)



(55)

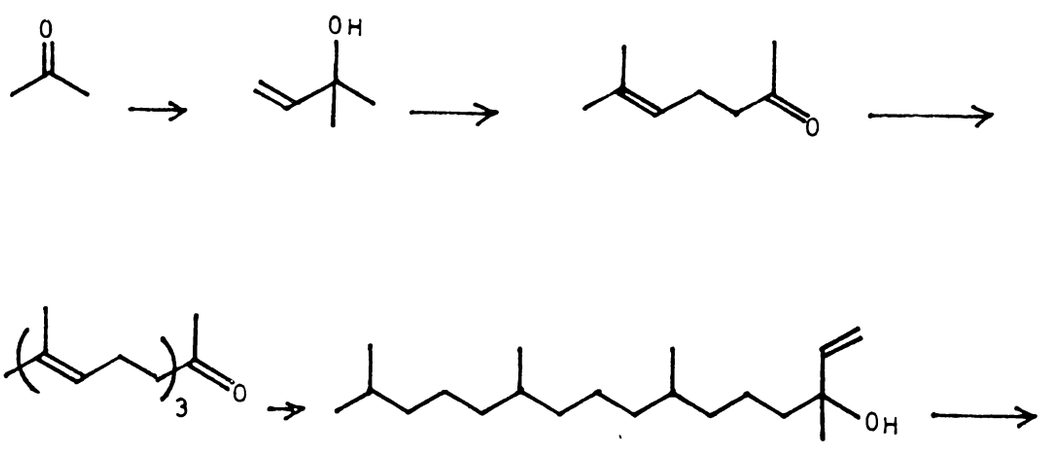


(53)

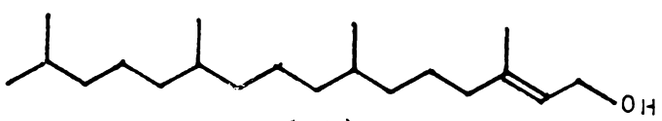
Multistep synthesis of dl phytol (52) starting from pseudoionone, citral and linalool have been reported[71-76].

Methods using acetone as the starting material are presently used to produce phytol and isophytol[77](55) as shown in Scheme XX.

Scheme XX



(55)



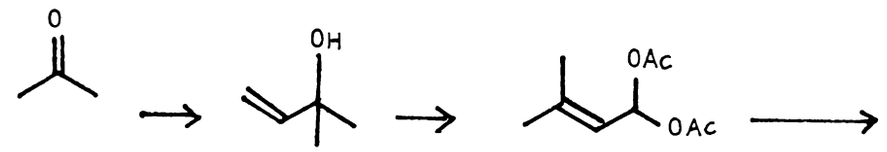
(52)

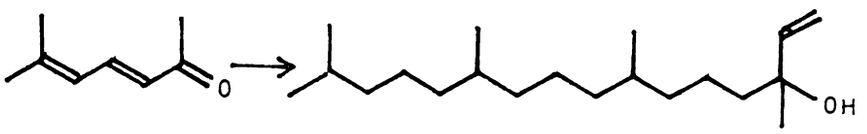
Analogues chain extension of acetone is performed using acetoacetic ester synthesis[78-81] or a reaction with diketene[82,83].

In another method acetone was ethynylated, the acetylenic alcohol formed was treated with acetic anhydride in presence of phosphoric acid and the resulting acetate was isomerised to obtain (56) which was then condensed with acetone to give (57).

Repetition of these reactions followed by hydrogenation afforded dl isophytol[84] (Scheme XXI).

Scheme XXI

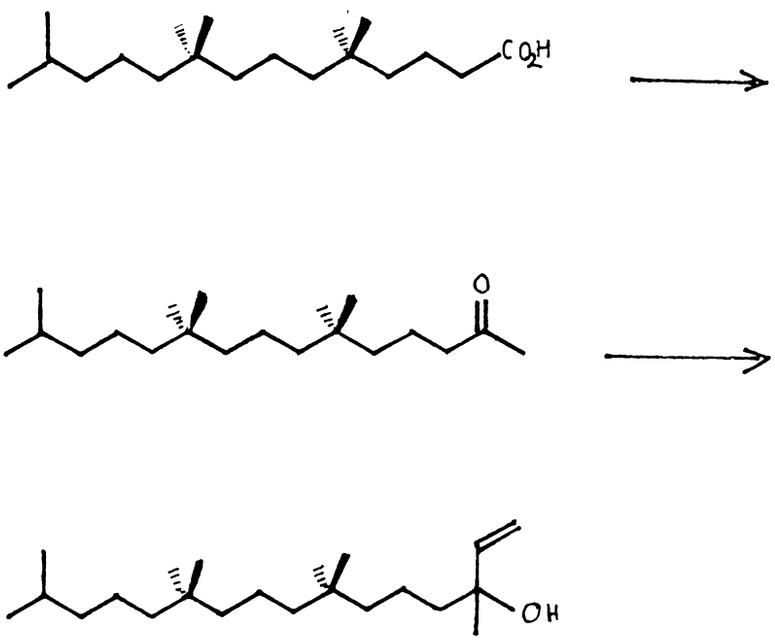




(55)

d-phytone (58) was prepared by treating (59) with sodium hydride in DMSO followed by refluxing in presence of aluminium amalgam. This was ethynylated and reduced to give d-isophytol[85] (Scheme XXII).

Scheme XXII



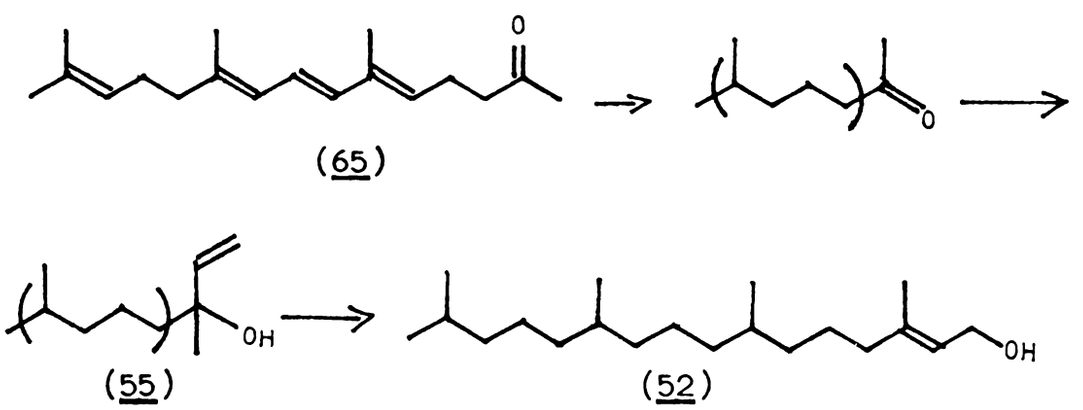
(55)

Lemongrass oil was refluxed with an aqueous solution of potassium carbonate to degrade the citral present in the oil to methyl heptenone. The product was then fractionated under reduced pressure. The top fraction

contained methyl heptenone together with hydrocarbons. It was then column chromatographed to get pure methyl heptenone. The keto group of methyl heptenone was then protected by ketalisation and oxidised using SeO_2 to get a mixture of allylic aldehyde and alcohol (60 and 61). This mixture was then reduced with $NaBH_4$ to the alcohol (61). (61) was brominated to (62) and then converted to the phosphonium salt (63). Wittig reaction of (63) with citral was conducted to get (64) which was deketalised to get the C_{18} ketone (65).

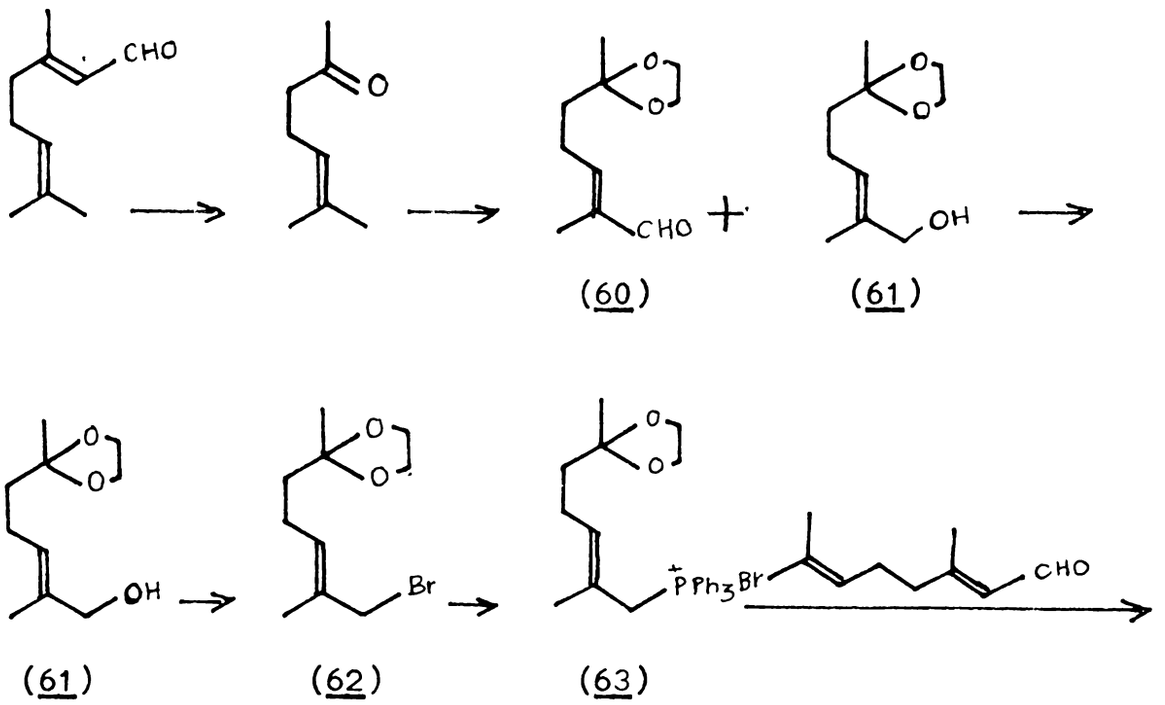
Since phytol is a key synthon in the synthesis of tocopherols and Vitamin K_1 a new approach for the synthesis of dl phytol using citral and methyl heptenone was undertaken (Scheme XXIII). Standard methods are available for the conversion of (65) to dl isophytol and dl phytol (Scheme XXIV).

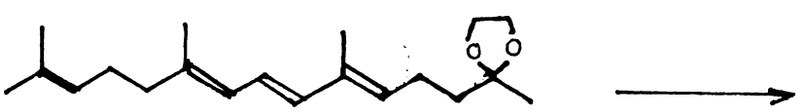
Scheme XXIII



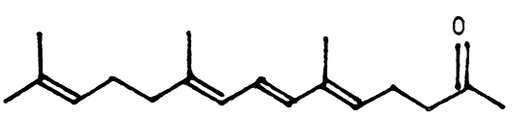
(65) (55) (52)

Scheme XXIV





(64)

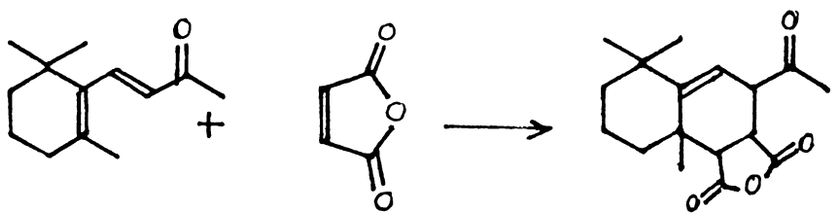


(65)

The C_{18} ketone (65) can also be used as an intermediate for the synthesis of tocopherols and tocotrienols (66) and Vitamin K_1 (54).

6.4 Studies on the reaction product of β-ionone and maleic anhydride

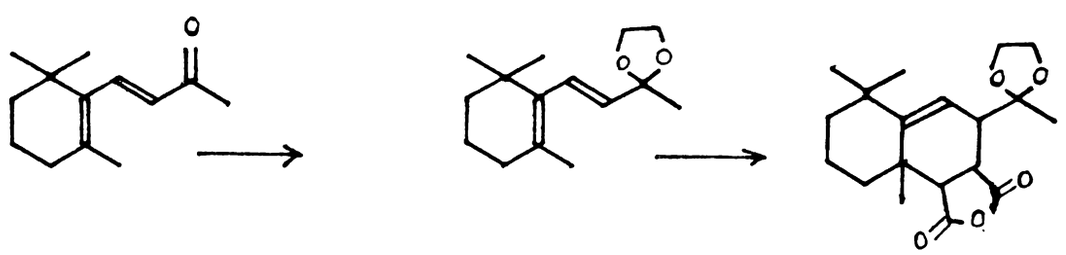
The Diels Alder product (67) if obtained from β-ionone and maleic anhydride finds use in the synthesis of compounds like Warburganal.



(67)

Scheme XXV was proposed for the synthesis of (68).

Scheme XXV



However this method did not work eventhough the reaction was conducted under varying conditions like, with and without solvent and under reflux. It was thought that since β-ionone which is supposed to act as the diene, contain an oxygen atom, the electrons may shift towards the oxygen atom thereby reducing the electron density available for the Diels Alder reaction to take place. It was planned to protect the carbonyl group by ketalisation prior to Diels Alder reaction. The ketal of β-ionone (69) was then refluxed with maleic anhydride for 15 hours and a solid product was obtained which was found to be polymeric in nature on spectral analysis. It was not able to predict the structure of the polymer formed,from the spectral data available. However the product formed was not a poly (maleic anhydride) since it was soluble in $CHCl_3$ and insoluble in water, whereas polymaleic anhydride is soluble in water and insoluble in $CHCl_3$. IR spectrum showed the presence of carbonyl group and the product did not melt even at 200°C. Further characterisation of the polymer formed was found to be difficult.

C H A P T E R VII

EXPERIMENTAL

7. Experimental

All gas chromatographic analysis were done on Hewlett Packard 5730 A chromatograph (6 ft. 10% SE-30 on chromosorb column, oven temperature 180° isothermal injection port temperature 200°C - detector temperature 250°C, FID detector, N_2 flow rate 40 minute) with a Hewlett Packard 3390 A Reporting Integrator. UV Spectra were taken on recording ultra violet spectrophotometer Hitachi Model 200-20. NMR spectra were recorded on a ^{1}H-FT NMR - Hitachi (60 MHZ, $CDCl_3$).

7.1 Novel Synthesis of Ionones

7.1.1 Pseudoionones

7.1.2 Requirements

Synthetic grade acetone supplied by E.Merck (India) and basic aluminium oxide-active - supplied by BDH were used as such. Citral was separated by column chromatography[69] from lemongrass (cymbopogon flexuosus) oil. Silicagel (Sisco, mesh 100-200) after activation at 100°C for 1 hour was used as the adsorbant for the chromatographic purification of the product obtained. Solvent grade hexane and ethyl acetate were used as the eluents for chromatography. For TLC silicagel supplied by E.Merck (India) was used.

7.1.3 Procedure

Pure citral (0.333 mol) dissolved in acetone (200 ml) was poured on basic alumina (100 gm) taken in a 1 litre R.B. flask equipped with a mechanical stirrer. It was then stirred continuously for 10 hours at room temperature. Aliquots were withdrawn at regular intervals and analysed by GLC and U.V.
It was found that 98% conversion of citral to pseudoionones occured in 10 hours. After the reaction was completed, the reaction mixture was filtered, alumina washed well with acetone and excess acetone recovered by distillation. The product obtained was further purified, by column chromatography. Fractions were collected and analysed by TLC, GLC and UV. Fractions containing pure pseudoionones were combined and solvent distilled off (water pump at 40°C) and the compound was obtained as a pale yellow oil bp 106-110°/2 mm.

Weight of pseudoionones obtained : 6.28(97%)

The product was then further characterised by GLC and spectroscopic analysis.

U.V. λ_{max}^{EtOH} 291.5 nm; 15,550; $n_D^{27^\circ}$ = 1.5260

NMR ($CDCl_3$)

1.22 (3H, S)

1.65 (4H, d)

1.85 (3H, S)

2.28 (6H, S)

5.1 (1H, broad)

6.1 (2H, m)

7.37 (1H, m)

The same experiment was conducted with stirring under reflux at 60°C. Under these conditions it was found that near 100% conversion of citral to pseudo-ionones occured within 4 hours as seen by GLC analysis.

7.2 Methyl Pseudoionone

7.2.1. Reagents

Ethyl methyl ketone, supplied by E.Merck(India) and basic aluminium oxide active supplied by BDH were used as such.

7.2.2 Procedure

Pure citral (0.0333 mol) was dissolved in ethyl methyl ketone (200 ml) and poured into basic alumina (100 gm) contained in a round bottomed flask, equipped

with a mechanical stirrer. It was then stirred at room temperature for 24 hours, aliquots were withdrawn at regular intervals and analysed by TLC and GLC. It was found that 90% conversion of citral to methyl pseudoionones has occured in 24 hours. Reaction mixture filtered, residue washed well with ethyl methyl ketone and ethyl methyl ketone removed by distillation. The crude product was then chromatographed on silicagel (Sisco Meshsize 100-200, after activation for 1 hour at 100°C) using hexane and ethyl acetate as eluents after proper drying in the ratio 9:1. The same experiment was repeated under reflux. At refluxing temperature the reaction was found to be almost complete in 5 hours to yield 95% methyl pseudoionone.

7.3 Cyclisation of Pseudoionones

7.3.1. Reagents

Synthetic grade ethyl acetate supplied by E.Merck (India), acidic alumina-active supplied by BDH and p-toluene sulfonic acid supplied by BDH were used as such.

7.3.2 Procedure

Pure pseudoionone 1 gm was dissolved in 40 ml ethyl acetate and poured on to acidic alumina (20 gm)

taken in a round bottomed flask equipped with a mechanical stirrer. It was then stirred at room temperature for 12 hours and analysed by TLC and GLC. Analysis showed the absence of any cyclisation.

The reaction was then repeated under reflux. Even then the result was negative. 5% solution of p-toluene sulfonic acid in ethylacetate (40 ml) was poured on to acidic alumina (20 gm) taken in a round bottomed flask and pure pseudoionone (1 gm) added to it. The reaction mixture was then stirred on a magnetic stirrer under reflux for 6 hours. Both GLC and TLC analysis showed the absence of any cyclisation of the pseudoionones.

The above experiment was repeated with acidic alumina (20 gm) soaked in a 10% solution of para toluene sulfonic acid in ethyl acetate (40 ml) at room temperature. Aliquots were withdrawn at regular intervals and analysed by TLC and GLC. GLC showed 93% conversion of pseudoionones to ionoones in 1 hour. The reaction mixture filtered and washed with ethyl acetate. The filtrate washed free of p-toluene sulfonic acid dried using anhydrous sodium sulphate and ethylacetate removed by distillation under reduced pressure (water pump). The crude product was then chromatographed on silicagel (Sisco-mesh size 100-200,

activated for 1 hour at 100°C) using hexane and ethyl acetate as eluents in the ratio 9:1 and checked by GLC.

Weight of ionones formed 0.935 g. (93.7%) GLC analysis showed the presence of α,β and γ-ionones in the ratio 44.7 : 4.8 : 42.2. The same experiment was repeated with acidic alumina (20 gm) soaked with a 20% solution of p-toluene sulfonic acid in ethyl acetate (40 ml), with stirring at room temperature using a magnetic stirrer. Aliquots were withdrawn at regular intervals of time and chekd by TLC and GLC. GLC analysis showed the cyclisation to be almost complete in about 20 minutes. The same work up in the above experiment was done and the crude product chromatographed and analysed by GLC.

Weight of ionones formed 0.95 g (95%)

The percentage of α,β and γ- Ionones formed are as given below:

$$\alpha = 54.4$$
$$\beta = 8.35$$
$$\gamma = 36.9$$

The above experiments were repeated by stirring under reflux also. Not much change was observed by refluxing the reaction mixture.

7.4 A new approach to the synthesis of β-Damascone

7.4.1 Synthesis of β-ionone

In a 1 litre 3 necked R.B.flask provided with a mechanical stirrer, a dropping funnel and a thermometer reaching the bottom of the flask, concentrated sulfuric acid (36.0 gm) was taken. The flask was cooled to -5°C and ethyl-acetate (15 ml) was gradually added under stirring at that temperature. Pseudo-ionone prepared by the condensation of citral and acetone using basic alumina[70] (15 g) was then added dropwise over 1.5 hour, taking care to see that the internal temperature of the reaction mixture did not exceed 5°C. After completion of the addition, stirring was continued for another 15 minutes and the reaction mixture was decomposed by pouring into a mixture of crushed ice and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined extract was washed successively with water, brine containing 1% sodium carbonate till alkaline and finally with brine till neutral. It was then dried over anhydrous sodium sulfate and the solvent removed. The residue was purified by distillation under vacuum.

Weight of β-ionone formed 12.75 g(85%)

The product was then further characterised by spectral data and GLC.

B.P. 92-96/2 mm

n_D^{270} 1.5165

U.V. λ_{max}^{EtOH} 296 nm (9700)
and 218 nm (7190)

These values showed the presence of 93% β-ionone.

It was then further purified by column chromatography on silicagel (Sisco meshsize -100-200, activated for 1 hour at 100°C) using hexane and ethyl acetate as eluents in the ratio 95:5.

UV λ_{max}^{EtOH} 297.5 nm

NMR ($CDCl_3$)
1.07 (6H, S), 1.55 (4H, m),
1.78 (3H,S), 2.09 (2H, n),
2.31 (3H,S), 6.11 (1H)
7.28 (1 H)

IR 2850, 1720, 1610,1360,1260,975

7.4.2 Reduction of β-ionone

7.4.2.1 Meerwein-Ponndorf-Verley Reduction

In a 500 cc R.B.flask were placed β-ionone (0.31 mol) and 1 M solution of aluminium isopropoxide in anhydrous isopropanol (300 cc). A short reflux

condenser was attached to the flask, but no water was circulated through the cooling jacket. To the top of the condenser a water cooled condenser was attached by using a distillation head. It was then refluxed at such a rate that 5 to 10 drops of the distillate (acetone) were collected per minute. The presence of acetone in the distillate was tested with 2,4-dinitrophenyl hydrazine reagent.

When the acetone test became negative, water was passed through the upright condenser and total reflux was maintained for 5 minutes. The water was again removed from the reflux condenser and the first 5 drops of the distillate was tested for acetone. When the negative test for acetone was obtained, most of the isopropyl alcohol was recovered and the reaction mixture hydrolysed with cold dilute hydrochloric acid and the cold suspension was mixed well by swirling to complete the hydrolysis. The mixture was then extracted with ether. Ether layer washed well with water, dried using anhydrous sodium sulfate and ether removed. The crude product was then characterised by TLC, GLC and UV.

Weight of crude product 50.4 gm (83%)

Analysis showed the presence of 3 products. It was then chromatographed on silicagel (Sisco-mesh size 100-200, after activation for 1 hour at

100°C)using hexane and ether as eluents in the ratio 95:5. Fractions with same Rf in TLC and same retention time in GLC were combined and solvent removed and weighed.

Percentage of I	35
Percentage of II	30
Percentage of III	5
Percentage of IV	28

Spectral data (UV and NMR) showed that components I and II were hydrocarbons; III β-ionone (unreacted) and IV β-ionol.

Characterisation of products

Component I

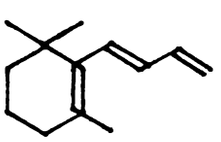
UV λ_{max}^{EtOH} 275 nm

NMR = 1.0 (3H, S)

1.1 - 2.1 (12H, m)

4.3 - 7.0 5H(olefinic)m

Hence the structure of this compound is assigned as (70).



(70)

Component II

U.V. λ_{max}^{EtOH} 236 nm

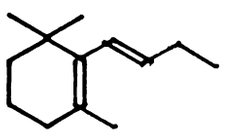
NMR

= 1.0 (3H,S)

1.05 - 2.1 (17H)

5.75 (2H, m)

Hence the structure of this compound is assigned as (71).



(71)

Component III

U.V. λ_{max}^{EtOH} 297.5 nm

NMR

= 1.07 (6H,S), 1.55 (4H,n),

1.78 (3H, S), 2.09 (2H, m),

2.31 (3H, S), 6.11 (1H,

7.28 (1H).

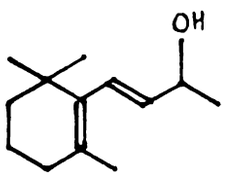
Hence the structure of this compound is assigned as B-ionone.

Component IV

NMR($CDCl_3$)

= 0.99 (6H,S), 1.32 (3H,d),
1.67 (3H,S), 1.73 (1H,S),
2.00 (2H, n), 4.40 (1H, m),
5.50 (1H, dd), 6.15 (1H.m).

Hence the structure of this compound as assigned as (72).



(72)

Analysis showed that only 28% of the products formed was β-ionol and the major products were hydrocarbons.

7.4.2.2 Sodium Barohydride reduction

Since the known procedure using alkaline $NaBH_4$ did not work for the reduction of β-ionone, a modified method was used.

Well powdered $NaBH_4$ (0.12 mol) was added with stirring to a solution of β-ionone (0.04 mol) in anhydrous methanol (50 ml). Stirring continued for 30 minutes using a magnetic stirrer at room temperature. Excess $NaBH_4$ removed by adding saturated NH_4Cl solution and then extracted with ether. Ether layer washed

with brine and dried using anhydrous sodium sulfate. Ether removed by distillation. TLC and GLC analysis of the product showed complete conversion of β-ionone to β-ionol.

Weight of β-ionol formed 7.53 gm(97%)

β-ionol was further characterised by NMR

H^1NMR ($CDCl_3$)

0.99 (6H,S), 1.32 (3H,d),
1.5 (2H, m), 1.67 (3H, S)
1.73 (1H, S), 2.00 (2H, n)
4.40 (1H, m), 5.50 (1H, dd)
6.15 (1H, m)

7.4.3 Acetylation of β-ionol

Acetic anhydride (24 ml) and pyridine(3 drops) were added to β-ionol (0.033 mol) contained in a 100 ml round bottomed flask was stoppered and kept for 24 hours with occasional shaking. TLC analysis showed the acetylation to be complete in 24 hours. The crude reaction mixture dissolved in ether and washed free of pyridine and excess acetic anhydride. Ether layer dried over anhydrous sodium sulfate and ether removed (water pump, 30°C) and weighed.

Weight of acetate formed 6.78 gm(95%)

The product was characterised by TLC, GLC and NMR.

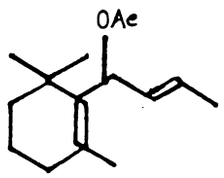
H^1NMR

0.98 (6H, S), 2.03 (3H, S)

1.1 - 2.0 (11H, m)

5.45 (2H, m), 6.10 (1H, m)

From NMR the structure of the acetate is assigned as (73).



(73)

7.4.4 Hydrolysis of iso-β-ionol acetate

Potassium carbonate (0.024 mol) dissolved in 25 ml methanol was added to iso-β-ionol acetate(0.02 mol) taken in a 100 ml conical flask. It was then stirred at room temperature using a magnetic stirrer for 1½ hour. Diluted with water, methanol removed (water pump), extracted with ether, ether layer washed free of alkali and then dried over anhydrous sodium sulphate. Ether removed (water pump) and weighed.

Weight of iso-β-ionol obtained 3.05(78%)

The product was then characterised by its H^1NMR.

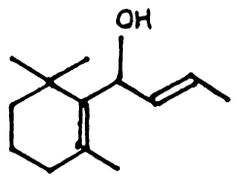
H^1 NMR

0.95 (6H, S), 1.23 (3H, S),

1.2 - 2.0 (9H, m), 1.88 (1H, S)

4.70 (1H, m), 5.70 (2H, m).

Hence the structure is assigned as (74).



(74)

7.4.5 Oxidation of Iso-β-ionol

0.1 mol Jones reagent was added slowly to a solution of iso-β-ionol (0.01 mol) in acetone, contained in a 100 ml Round bottomed flask, with stirring at room temperature using a magnetic stirrer. Addition of Jones reagent continued till the colour of the reagent persists. Excess Jones reagent was destroyed by adding a few drops of iso-propanol. The reaction mixture diluted with water, acetone removed (water pump) extracted with ether and ether layer washed free of acid and dried over anhydrous sodium sulphate. Ether removed and weighed.

Weight of crude product 1.23 gm(64%)

The product was then characterised by I.R., U.V. and NMR.

U.V. λ_{max}^{EtOH} 273 nm and 227 nm

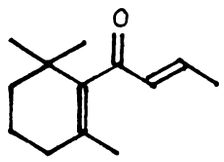
I.R. 2850, 1620, 1675, 970

H^1 NMR

1.0 (6H, S), 1.50 (3H), 1.92 (3H, m),

1.1 - 2.2 (6H, m), 5.6 - 6.3 (2H, m)

Hence the structure is assigned as (46).



(46)

7.4.6 Oxidation of iso-β-ionol acetate

The hydrolysis and oxidation of iso-β-ionol acetate was tried in a single step.

Jones reagent(0.1mol)was added under stirring to a solution of iso-β-ionol acetate (0.02 mol) in acetone taken in a round bottomed flask, at room temperature. Addition continued till the colour of the reagent persisted. Excess reagent was destroyed by adding isopropanol. The reaction mixture was diluted with water and acetone removed (water pump). It was the extracted with ether, ether layer washed free of acid, dried using anhydrous Na_2SO_4, ether removed and purified by column chromatography on silicagel (Sisco-Meshsize 100-200, activated for 1 hour at 100°C) using hexane and ethyl acetate as eluents in the ratio 9:1.

Weight of product obtained - 2.01 gm(52%)

The product was then characterised by its U.V., I.R. and NMR.

U.V λ_{max}^{EtOH} 273 nm, 227 nm

I.R. $CHCl_3$(solution) 2850 cm^{-1}, 1620 cm^{-1}, 1675 cm^{-1}, 970 cm^{-1}

H^1NMR ($CDCl_3$)

= 1.0 (6H, S), 1.50 (3H), 1.92 (3H, m), 1.1 - 2.2 (6H, m) 5.6 - 6.3 (2H, m)

7.5 Synthesis of dl-Phytol

7.5.1 Preparation of Methyl Heptenone

A dilute aqueous solution of potassium carbonate was added to lemongrass (Cymbopogon flexuosus) oil (65 g) contained in a round bottomed flask. It was then stirred under reflux on an oil bath for 7 hours. The reaction mixture cooled, washed free of alkali, dried over anhydrous sodium sulphate and chloroform distilled off. The crude product obtained was then fractionated under vacuum. TLC analysis of the first fraction showed it to be a mixture of hydrocarbons and methyl heptenone. It was then column chromatographed on silicagel (Sisco-Meshsize 60-120, after activation for 1 hour at 100°C) using hexane and ether as eluents in the ratio 98:2. Fractions collected in small portions and fractions having same Rf in TLC and same retention time in GLC were combined, solvent removed and weighed.

Weight of pure methyl heptenone obtained : 35.01 g(87%)

7.5.2 Protection of the keto group of Methyl Heptenone by Ketalisation

Ethylene glycol (75 ml) and thiophene free benzene (200 ml) were placed in a 500 ml R.B.flask equipped with a Dean-Stark apparatus and refluxed on a water bath to remove water present. When no more water collects in the Dean-Stark apparatus,

methyl heptenone (0.08 mol) and a pinch of p-toluene sulfonic acid were added to the flask and refluxed for 6 hours. The benzene layer separated, washed with water to remove excess ethylene glycol and p-toluene sulfonic acid. It was then dried over anhydrous sodium sulphate, benzene distilled off and weighed. The crude product was then purified by column chromatography over silicagel (Sisco-Meshsize 100-200, after activation for 1 hour at 100°C) as adsorbant and hexane and ethyl acetate in the ratio 95:5 as eluent.

Weight of pure product obtained = 12.02 g(88%)

The ketal was further characterised by NMR.

H^1NMR ($CDCl_3$)

1.65 (6H, d)
1.33 (3H, S)
1.5 - 2.4 (4H, m)
3.95 (4H, S)
5.25 (1H, m)

7.5.3 Allylic Oxidation of the Ketal of Methyl Heptenone[87]

SeO_2 (0.04 mol) dissolved in dry ethyl alcohol was added slowly to the ketal of methyl heptenone (0.03 mol) in dry ethyl alcohol with stirring under reflux. Stirring under reflux continued for 2 hours, the reaction mixture analysed by TLC, diluted with water, concentrated in

vacuum, filtered and extracted with ether. The ether layer washed with $NaHCO_3$, sodium chloride and then with water. It was then dried over anhydrous sodium sulphate and ether removed. TLC analysis showed the product to be a mixture of aldehyde and alcohol.

Weight of crude product = 4.7 g (84%)

7.5.4 Reduction of the mixture of Aldehyde and Alcohol

To a solution of the mixture of aldehyde and alcohol (0.025 mol),in anhydrous methanol well powdered sodium borohydride (0.050 mol) added with stirring at room temperature and stirring continued for 1 hour. Excess $NaBH_4$ decomposed by adding a saturated solution of NH_4Cl, extracted with ether, ether layer washed with NaCl and then dried over anhydrous sodium sulphate. Ether removed by distillation and the product obtained purified by colum chromatography using silicagel (Sisco-Meshsize 100-200, after activation for 1 hour at 100^oC) as adsorbant and hexane and ethyl acetate as eluents in the ratio 9:1.

Weight of pure alcohol obtained = 2.9 g (68%)

7.5.5 Bromination of the Allylic alcohol

To a stirred mixture of the allylic alcohol (0.02 mol) and pyridine (0.1 ml) in dry ether (50 ml) contained in a three necked R.B.flask was added PBr_3 (2.33 cc) in dry ether (20 ml) at 0^oC over 1 hour. Stirring at 0^oC continued for 5 hours. The reaction mixture was poured into ice water, extracted with ether washed free of pyridine and PBr_3, dried using anhydrous sodium sulphate and concentrated in vacuum.

Weight of Bromide obtained = 2.08 g (70%)

7.5.6 Preparation of the Triphenylphosphonium salt of the Allylic Bromide

The allylic bromide (0.007 mol) was added to triphenylphosphine (0.007 mol) dissolved in dry benzene, under stirring. Stirring continued for 48 hours and solvent removed under vacuum.

Weight of product formed = 3.00 g (80%)

7.5.7 Synthesis of the C_{18} ketone

7.5.7.1 Preparation of Butyl Lithium

Fine lithium shavings (0.025 mol) were taken in 5 ml ether in a 100 ml three necked R.B.flask. It was cooled to -15^o using a ice-salt mixture. A few drops

of n-butyl bromide was added. A cloudiness showed the intitiation of the reaction. The remainder of n-butyl bromide (0.01 mol) was added in 30 minutes. When all the lithium shavings were dissolved, it was stirred for further 10 minutes at this temperature.

7.5.7.2 Preparation of phosphorane

n-Butyl lithium solution and 20 ml anhydrous ether were stirred. The phosphonium salt was added to it under stirring. Scarlet red colour appeared. Stirring continued for 15 minutes. Colour of the reaction mixture slowly changed to rose.

7.5.7.3 Condensation

Citral (0.011 mol) in ether was added dropwise to the phosphorane under stirring at room temperature. Stirring continued for 5 hours at room temperature. The reaction mixture was then added to cold water and extracted with ether. Ether layer washed with brine and then with water. It was then dried over anhydrous sodium sulphate, solvent removed under vacuum and weighed.

Weight of crude product = 2.30 g (78%)

The crude product was then purified by column chromatography over silicagel (Sisco-Meshsize

100-200) using hexane as eluent. It was then identified by NMR.

H^1NMR ($CDCl_3$)

1.33 (3H, S) ; 1.65 (6H, d)

1.88 (3H, S) ; 2.05 (3H, S)

3.95 (4H, S) ; 1.1 - 2.4(6H, m)

5.25 (1H,m) ; 5.9 - 7.7 (4H, m)

7.5.7.4 Hydrolysis of Ketal

The ketal dissolved in acetone was stirred at room temperature with an excess of p-toluene sulfonic acid overnight. The reaction mixture was checked by TLC. Water was added to it and acetone removed in vacuum, extracted with ether, ether layer washed (Sodium bicarbonate, brine and water) and dried over anhydrous sodium sulfate. Solvent removed and the product purified by column chromatography to give 56% of the C_{18} ketone. It was then characterised by H^1NMR.

H^1NMR ($CDCl_3$)

1.65 (6H, d); 1.88 (3H, S)

2.33 (3H, S); 2.05 (3H, S)

1.1 - 2.4 (6H, m)

5.25 (1H, m); 5.9 - 7.7 (4H, m)

7.6 Studies on the reaction product of β-ionone and Maleic Anhydride

7.6.1 Diels-Alder adduct of β-ionone and Maleic Anhydride

Equimolar mixture of β-ionone and maleic anhydride were heated with and without solvent at different temperatures (40,60,80 and 100°C) for different intervals of time (4,6 and 10 hours). It was found that no reaction was taking place. Then the keto group of the β-ionone was protected and treated with maleic anhydride.

7.6.2 Ketalisation of β-ionone

Ethylene glycol (30 ml) and thiophene free benzene (175 ml) were taken in a 500 ml R.B.flask fitted with a Dean-Stark apparatus and a reflux condenser. It was then refluxed on a water bath to remove water present. When no more water collects in the Dean-Stark apparatus, β-ionone (0.05 mol) and a pinch of p-toluene sulfonic acid were added to the flask and refluxed for 6 hours, the benzene layer separated, washed with water to remove excess ethylene glycol and p-toluene sulfonic acid. It was then dried over anhydrous sodium sulphate, solvent removed under vaccum to give 10.47 g (88%) of the crude product.

It was then purified by column chromatography using silicagel (Sisco-Meshsize 100-200) as adsorbant and a mixture of hexane and ethyl acetate as eluent in the ratio 95:5. It was then identified using NMR.

H^1NMR ($CDCl_3$)

1.0 (6H, S)

1.2 - 2.0 (12H, m)

3.98 (4H, S)

5.50 (2H, m)

7.6.3 Preparation of the reaction product of the ketal of β-ionone with Maleic Anhydride

Equimolar mixture of the ketal of β-ionone and maleic anhydride were heated on an oil bath for 14 hours and then filtered. The product was then chromatographed on silicagel (Sisco Meshsize 60-120) using a mixture of hexane and ethyl acetate as eluent in the ratio 9:1 to give a substance melting above 200°C.

Spectral data (NMR and IR) of the product showed it to be not the expected product. It was not possible to predict the structure from the data available.

C H A P T E R VIII

RESULTS AND DISCUSSION

8.1 Analysis of Lemongrass Oil

8.1.1 A New Method of Estimation of Citral in Lemongrass Oil

A new method for the estimation of citral in lemongrass oil has been developed using column chromatographic technique. With a ratio of silicagel to lemongrass oil 35:1 and with hexane and ether as eluents in different ratios it is now possible to estimate the correct percentage of citral present in lemongrass oil by separating all the citral present in pure form. This method of estimation is found to be superior compared to the existing methods. Since in the bisulphite adducting, neutral sulphite adducting and in hydroxyl amine methods all the carbonyl compounds other than citral present in lemongrass oil are also getting adducted along with citral and the values obtained are generally much higher than the actual citral content. Colorimetric method is found to be tedious and solutions of citral with known strength are needed for the estimation. In almost all the methods available citral is lost during estimation. However no loss happens in the newly developed technique, since in this method, physical separation of citral is only involved. Also all other components are recovered as mixture of

hydrocarbons (limonene-myrcene and dipentene), mixture of geranyl acetate, methyl heptenone, citronellal and n-decyl aldehyde and mixture of alcohols (geraniol, nerol, methylheptenol, linalool and citronellol).

8.1.2 A New Method of Separation of Citral from Lemongrass Oil by Physical Separation of Citral

The column chromatographic method for the estimation of citral in lemongrass oil was extended for the separation of citral in lemongrass oil. Commonly used method of separation of citral in industries is by fractional distillation of the oil under reduced pressure. Even when efficient fractionating columns are used citral of only about 95% purity is generally obtained. It is found difficult to remove components like geraniol, nerol etc. which differ only by few ^{o}C in their boiling points with that of citral. In chemical methods for the separation of citral, purity of citral obtained is generally poor and aldehydes like citronellal and n-decyl aldehyde and ketones like methyl heptenone present in lemongrass oil are generally present in the citral separated. In the chemical methods of separation a loss of 10-15% of citral is usually observed. There are also possibilities for the rearrangement of citral. In the bisulphite adducting

methods, the bisulphite is reported to get added on to the double bonds also and if the bisulphite get added on to the terminal double bond citral cannot be regenerated. In the column chromatographic method, since citral is separated physically without recourse to any chemical reaction, the possibilities for the rearrangements are minimal. By this newly developed method, citral (of 99+% purity - by GLC) in near quantitative yield is obtained by a single column chromatographic operation using silicagel (Sisco Mesh-size 100-200) to lemongrass oil in the ratio 10:1 and hexane and isopropanol as eluents in the ratio 95:5. Since the adsorbant and eluents can be recycled after proper refining, this method of separation of citral can have industrial value.

8.1.3 Total Analysis of Lemongrass Oil

Total analysis of lemongrass oil (Cymbopogon flexuosus) oil was attempted by the separation of all components present in lemongrass oil by column chromatography. However it was found difficult to separate all the components present in lemongrass oil by column chromatography. Lemongrass oil was separated into four fractions namely hydrocarbons, geranylacetate together with carbonyl compounds other than citral, pure citral and alcohols on a column using silicagel (Sisco Meshsize 100-200) as adsorbant in the ratio 1:10 and

hexane and isopropanol as eluents in different ratios. The mixed fractions were then rechromatographed. With a ratio of substance to silicagel 1:50 and using hexane as eluent it is found possible to separate myrcene from the mixture of hydrocarbons (myrcene, limonene and dipentene). 7% of lemongrass oil is found to be mixture of hydrocarbons (4.5% myrcene and 2.5% mixture of dipentene and limonene). The second fraction obtained by the column chromatography of lemongrass oil is found to contain geranyl acetate, citronellal, n-decyl aldehyde and methyl heptenone. From this mixture geranyl acetate and methyl heptenone can be separated in pure form. 8% of lemongrass oil is found to be a mixture of these components, 70% of which is geranyl acetate (i.e. 5.6% of lemongrass oil) and methyl heptenone accounts for 1.44% of lemongrass oil.

The last fraction obtained on column chromatography of lemongrass is found to be mixture of methyl heptenol, linalool, nerol, geraniol and citronellol. This accounts for 12.33% of lemongrass oil. Linalool was separated in pure form this mixture using column chromatography. This accounts for 3% of lemongrass oil.

8.1.4 Chromatographic Analysis of Noncitral Portion and Ionone tops of Lemongrass oil.

Analysis of the non-citral portion of lemongrass oil and ionone tops obtained by the fractionation of

lemongrass oil after condensing with acetone was conducted using column chromatography. 26.22% of non-citral portion of lemongrass oil was a mixture of hydrocarbons and 43.07% alcohols. 12% of ionone tops were found to be hydrocarbons and 72.35% alcohols.

8.1.5 Separation of cis trans Isomers of Citral

The separation of cis trans isomers of citral to neral and geranial was tried using colum chromatography. Complete separation of these two isomers has not yet been reported. Isolation of one isomer using chemical methods were reported with the loss of the other isomer. Separation of these two isomers were also reported through their semicarbazones. However the purity of these products have not been critically examined as much of this work had been done during the pre-glc period. By fractional distillation using spinning band column it was reported recently that out of the six fractions collected the last fraction was found to be 95% geranial. On a silicagel column with a ratio of 1:100 (substance : adsorbant) using hexane and isopropanol as eluents it is possible to separate citral into fractions of pure neral, mixture of neral and geranial and pure geranial in the following ratio 25:45:30. Separation of the two isomers were also tried as their corresponding alcohols (geraniol and nerol). Citral was reduced to mixture of geraniol

and nerol using aluminium isopropoxide in anhydrous isopropanol. GLC analysis of the reduction product showed that 20% of the product formed was hydrocarbons. It was then found possible to separate this mixture to hydrocarbons, pure nerol, mixture of geraniol and nerol and pure geraniol in the ratio 20:5:50:25. Hence no advantage was observed by reduction of citral before separation, and in this case substantial portions of the aldehyde are converted to rather useless hydrocarbons.

8.2 Industrial uses for the Components of Lemongrass Oil

8.2.1 A Novel Synthesis of Pseudoionone from Citral and Acetone using Alumina

A novel method for the synthesis of pseudoionone using citral and acetone was achieved with basic alumina as the condensation catalyst. Pure pseudoionone (99% purity by GLC) in 93%, yield was obtained using citral, acetone and basic alumina in the ratio 1:40:20. Since this method is a simple method requiring no neutralisation of excess alkali as in the case of other methods and since alumina and excess acetone can be recovered and reused this method can have commercial application.

8.2.2 A Novel Synthesis of Methyl Pseudoionone

Methyl pseudoionones were also prepared from citral and ethyl methyl ketone using the above procedure.

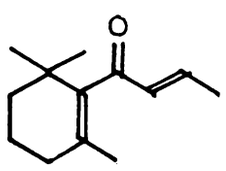
By this process 95% conversion of citral to methyl pseudoionones was achieved in 5 hours.

8.2.3 Novel Cyclisation of Pseudoionone to Ionones

Pseudoionones were cyclised using acidic alumina soaked with p-toluene sulfonic acid. 20% solution of p-toluene sulfonic acid in ethyl acetate was found to give the best results. With a ratio of pseudoionones, acidic alumina and 20% solution of p-toluene sulfonic acid in ethyl acetate 1:20:40, 98% conversion of pseudoionones to ionones was achieved in 30 minutes. In this method the major products obtained are α and γ- ionones and β-ionone is found only to the extent of 4-6%.

8.2.4 A Novel Approach for the Synthesis of β-Damascone from β-ionone

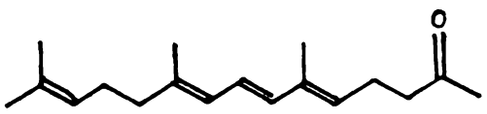
β-Damascone (I) was synthesized from β-ionone. β-ionone was reduced and the β-ionol obtained was rearranged to iso-β-ionol via acetylation. It was then oxidised to β-Damascone.



(I)

8.2.5 A New Approach to the Synthesis of Phytol

The synthesis of C_{18} ketone (II), a well established key intermediate, for the synthesis of phytol, tocopherols and tocotrienols was achieved using methyl heptenone and citral.



(II)

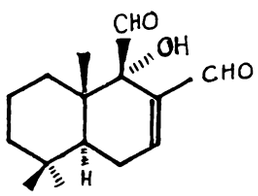
8.2.6 Studies on the Meerwein-Ponndorf-Verley reduction of α-β unsaturated Carbonyl Components

α-β unsaturated carbonyl components like citral and β-ionone were reduced by Meerwein-Ponndorf-Verley reduction. It was found that hydrocarbons were formed in substantial quantities, when this reduction was done on citral, 20% of the product formed being hydrocarbons. It was found that when the conjugation increases the percentage of hydrocarbon formed also increases. Thus in the case of β-ionone 65% of the product formed was found to be hydrocarbons.

8.2.7 Diels Alder adduct of β-ionone and maleic anhydride

Attempt was made to prepare the Diels-Alder adduct of β-ionone with maleic anhydride which is a good

synthon in the synthesis of Warburganal (III). Since the Diels-Alder reaction was unsuccessful, the keto group of β-ionone was protected and then the reaction was repeated. A solid product was obtained. Spectral analysis showed that the product formed was not the expected one.



(III)

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A NEW METHOD OF ESTIMATION OF CITRAL IN LEMON GRASS OIL BY PHYSICAL SEPARATION OF CITRAL.

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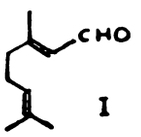
Abstract

At present the usual method of estimation of citral in lemon grass (C i m b o p o g e n f l e x u o s u s) oil is by sodium bisulphite adducting. However this method cannot be expected to give correct value since other aldehydes and methyl ketones, known to be present in lemon grass oil will also get adducted. A new method of estimation for citral is now developed which avoids this defect, by physically separating citral quantitatively in pure form from lemon grass oil samples by column chromatography. This method yields very reliable results, since the citral present is physically isolated quantitively from the oil in pure form. The above method involving the use of no chemical, can also be adopted as an excellent method, superior to all the other methods known for the isolation of pure citral. Hence this method can be of commerical value for the production of pure citral.

1. Introduction

Lemon grass oil (obtained by steam distillation of lemon grass (C i m b o p o g e n f l e x u o s u s) contains generally about 70-80% citral - a mixture of geranial (citral-a) (I) and neral (citral-b) (II) roughly in the ratio of 5:3.



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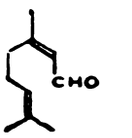


II

Various methods are known for the estimation of citral in lemon grass oil. Of these by far the most commonly used method is adducting using sodium bisulphite. Since sodium bysulphite is known to adduct with aldehyde and methyl ketones, in the above method apart from citral, aldehydes like citranellal, n-decyl aldehyde and methyl ketones like methyl heptenone, known to be present in lemon grass oil will also get adducted. Moreover the sodium bisulphite is also known to add to the ethylenic double bonds. Hence this method always gives a higher value. With a view to estimate the correct percentage of citral in lemon grass oil by physical separation of citral quantitatively in pure form, a method was developed for separation of citral using column chromatographic technique.

Preliminary experiments were done using neutral alumina as adsorbant and hexane as eluent. Even though it was possible to separate all the hydrocarbons using this system, it was found that on prolonged column chromatography, the column developed heat and the chromatography could not be done satisfactorily. This can be attributed to the heat of

polymerisation of the highly reactive α-β unsaturated aldehydes namely citral-a and citral-b on the alumina column. Hence further chromatographic studies were undertaken using the milder adsorbant silicagel using hexane as eluent. Preliminary studies gave promising results. After repeated column chromatographic studies it was found possible to isolate citral quantitatively in pure form (purity 99+% by GLC) and thus calculate the correct percentage of citral in lemon grass oil samples.

Different varieties of lemon grass oil obtained from different regions of India (RRL-Jammu, RRL-Bhubaneswar, RRL-Jorhat, Odakkali), a sample from Bhuttan and a commerical sample from Cochin were analysed by the newly developed column chromatographic technique. By this method lemon grass oil could be separated into four fractions, namely hydrocarbons, carbonyl compounds other than citral, citral and alcohols.

2. Experimental

2.1 All gas chromatographic analysis were done on Hewlett Packard 5730A Gas Chromatograph with Hewlett Packard 3390A Reporting Integrator. (Column-6ft, 10% SE-30 on chromosorb, oven temp: 150°C isothermal, injection temp: 200°C, Detector temp: 250°C, N_2, (40 ml/minute) FID-detector).

2.2 Complete removal of solvents was accomplished using Buchi EL-130 Rotavapor.

2.3 Solvents used were dried before use.

2.4 Procedure (Sample - RRL-16 Jammu)

Column diameter	= 25 mm (ID)
Column adsorbant	= Silica gel - BDH (meshsize 60-120)
Ratio of substance to adsorbant	= 1:35
Weight of substance taken	= 4.160 gm

Fractions were collected, analysed (TLC), solvent removed and weighed (Table 1).

All the samples were analysed using the above procedure. For comparison citral content was also estimated by the standard sodium bisulphite adducting method (Table 2).

2.5 Typical Procedure for Estimation of Citral in Lemon Grass Oil by Sodium Bisulphite Adducting (Guenther, 1972)

10 cc of lemon grass oil taken in a Cassia flask (150 cc) was treated with 75 cc of freshly prepared saturated aqueous solution of sodium bisulphite. The adduct thus obtained, after thorough shaking was made into a solution by immersing the flask in boiling water. A further addition of 25 cc of saturated bisulphite solution was made. The flask was then kept undisturbed to allow the unreacted oil to rise to the surface. After cooling the oil layer was brought up to the top of the graduation mark by adding sodium bisulphite solution and the amount of unreacted oil measured. The citral content was calculated as follows.

Percentage of citral = 10 (10-No: of cc of unreacted oil).

3. Conclusion

As expected the values obtained by the column chromatographic method are lower by 2 to 9 units but in most cases 7 to 8 units than that obtained by the sodium bisulphite adducting method.

Because of the possibility of the presence of aldehydes other than citral and methyl Ketones in lemon grass oil samples, only the present method can be taken as a reliable method for estimating citral.

This method can also be used for quantitative separation of citral in pure form. Other methods used so far have many disadvantages. Since this method of separation of citral in pure form is of commercial value, this will form the subject of a future patent.

4. Discussion

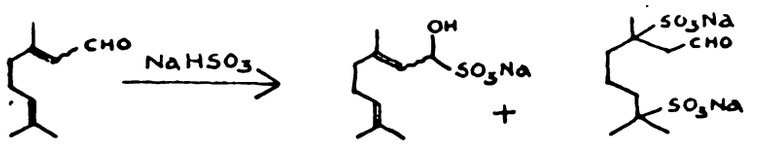
Methods of separation of citral

Chemical Methods

(Didge (1936), Dodge (1940), Gildmeister et al., Tiemann et al., (1893, 1898).

The separation of citral by adducting with sodium bisulphite and then regenerating citral by decomposing the adduct with either acid or alkali has many disadvantages. It will be very difficult to obtain pure citral since other aldehydes and methyl ketones also get adducted. In the decomposition if acid (hydrochloric acid) is used there are chances of citral getting cyclised and also polymerised and if alkali is used, instead, there will be 10 to 15% loss in the recovery. The loss is due to the formation of cyclic - bisulphite compound in presence of alkali from which recovery of citral is found to be very difficult.

Since citral is a mixture of cis-trans α - β unsaturated aldehydes, there are complications in bisulphite adduct formation. In addition to the normal bisulphite compound, compound containing 2 mols of sodium bisulphite are also reported. i.e. sodium bisulphite will also add on to the ethylenic double bonds as shown below.



This dihydrosulfonic derivative can exist in two forms: a labile form and a stable form and citral can no longer be regenerated from the stable form.

Physical Separation

Fractional distillation

Another method to separate citral from lemon grass oil is by fractional distillation. However it is very difficult to get pure citral by fractionation, even when the fractionation is conducted at different conditions like very low pressure etc. Moreover part of the citral will get polymerised when heated for a long time. It is also difficult

to remove the components like geraniol, nerol etc. differing only by a few degrees in their boiling points with that of citral. Hence only an enrichment of citral is generally possible (purity 95+% by GLC).

Column Chromatography

In the Column Chromatographic method, since no chemical reagents are used, the possibility of rearrangements of citral is minimised. Since this is a non destructive method, citral is obtained in pure form, and hence this procedure can be used as a method of separation of citral quantitatively from samples of lemon grass oil.

The preliminary work indicated the possibility of extending this method to oils containing related aldehydes like citronellal. Work is in progress to standardise this method in the case of such oils also.

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Table-1 - Details of column chromatographic technique

Sl. No.	Solvent used	Vol. of solvent used	Weight of substance	Method of detection	Remarks	%
1.	hexane	1000 ml	0.255 gm	TLC	Hydrocarbons	6.13
2.	98% hexane	1500 ml	0.300 gm	TLC	Carbonyl compounds other than citral	7.21
3.	95% hexane 5%	2000 ml	3.170 gm	TLC & GLC	Citral	76.21
4.	100% ether	750 ml	0.400 gm	TLC	Alcohols	9.62

Total weight recovered = 4.125 gm

Percentage recovery = 99

Table-2 - Analysis of different strains of lemon grass oil

Sl. No.	Samples	% of citral		Excess of citral by adducting
		1. Column Chromatography	Sod. bisulphite adducting	
1.	Jammu-RRL-16	76.2	84.0	7.8
	Bhubaneswar			
2.	O.D. - 440	73.0	80.0	7.0
3.	O.D. - 19	67.64	76.0	8.34
4.	RRL - 13	65.0	72.0	7.0
5.	RRL - 16	72.28	75.0	2.72
6.	SD - 68	71.0	80.0	9.0
	Jorhat			
7.	RRL - J.No.1	68.85	-	-
8.	RRL - J.No.2	71.09	-	-
9.	RRL - J.No.3	72.33		-
10.	RRL - J.No.4	73.26	-	-
11.	RRL - J.No.5	69.97	-	-
	Odakkali			
12.	O.D. - 440	90.00	94.0	4.0
13.	O.D. - 408	75.0	84.0	5.0
14.	O.D. - 19	72.8	76.0	3.2
15.	Commercial sample from Cochin	70.25	78.0	7.75
16.	Bhutan	68.98	76.0	8.0